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AUTHORS' ERRATA AND EMENDATIONS

Page 1, footnote 1, line 8, "*riscose*" should be "*viscosa*", and, line 9, "CAUSAPAS" should be inserted between "PLANTAS" and "POR."

Page 66, last line, "(p. 64)" should be "(p. 63)."

Page 128, lines 1 and 2, hyphens should be deleted after 25, 50, and 100.

Page 150, table 1, last column, third line from bottom should be "8 S, 92 SR" and bottom line should be "63 S, 37 SR."

Page 163, third paragraph, line 3, "*Phleum praterise*" should be "*Phleum pratense.*"

Page 409, footnote 1, formula should read

$$\sigma = \pm\sqrt{\frac{\Sigma d^2 f}{(n-1)}}.$$

Page 422, table 5, next to last column, under *P*, "0.5" should be "0.05."

Page 459, reference 2, "average" should be "acreage."

Page 477, reference 16, "Berücktsichtigung" should be "Berücksichtigung."

Page 520, legend for figure 6, fourth line, "*B*" should be "*E.*"

Page 521, line 17, "only" should be inserted between "though" and "three."

Page 535, third line from bottom, "equation (3)" should be "equation (4)."

Page 540, line 7, "equation (5)" should be "equation (6)."

Page 551, third paragraph, line 8, "monogram" should be "nomogram."

Page 567, table 5, last column, symbol for cod-liver supplement in experiment no. 2 "F" should be "E."

Page 612, seventh line from bottom, "70°" should be "75°."

Page 658, sixteenth line from bottom, "if their material" should read "in their material."

Page 705, footnote, "page 14" should be "pages 706 and 707."

Page 757, first formula should read

$$\sigma_{1-2} = \sqrt{\frac{\Sigma D_2 - (\Sigma D)\ (M_D)}{N(N-1)}}.$$

Page 793, thirteenth line from bottom "changed to" should be "charged to."

Page 802, twenty-third line from bottom, "2,000 kg" should be "2 kg."

Page 912, next to last line, "hardening" should be "hardened."

Page 926, complete reference by adding "54:899–917." after Jour. Agr. Research.

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SWEET ORANGE FRUIT SCAB CAUSED BY ELSINOË AUSTRALIS [1]

By AGESILAU A. BITANCOURT, *subdirector de biologia vegetal, Instituto Biologico de São Paulo (Brazil)*, and ANNA E. JENKINS, *associate mycologist, Division of Mycology and Disease Survey, Bureau of Plant Industry, United States Department of Agriculture* [2]

INTRODUCTION

A study of the sweet orange fruit scab, caused by *Elsinoë australis* Bitancourt and Jenkins (*5*),[3] as yet identified only in South America, is reported in this paper. The sour orange scab,[4] caused by *Elsinoë fawcetti* Bitancourt and Jenkins (*4*), and the Australian citrus scab, caused by *Sphaceloma fawcetti scabiosa* (McAlp. and Tryon) Jenkins (*23*), are almost never found (*23*, *40*) on the sweet orange (*Citrus sinensis* Osbeck), and when present do little or no damage.

Inasmuch as *Sphaceloma perseae* Jenkins (*22*), the causal fungus of scab of avocado (*Persea americana* Mill.), is morphologically similar to the conidial stage of *Elsinoë fawcetti*, having at first been confused with it, this organism was included in most of the comparisons of *E. australis* with *E. fawcetti*. The comparisons served to demonstrate that *E. australis* is also distinct from *S. perseae*, and they confirmed the conclusion (*22*) that *S. perseae* and *E. fawcetti* are two different species. Only a brief report of the comparisons with *S. perseae* is given.

Experiments on the control of sweet orange fruit scab have not been included in the present study. Wright and Moreira (*41*), however, have shown by a series of spraying experiments that the disease can be controlled by essentially the same methods as those employed for sour orange scab.

---

[1] Received for publication Apr. 27, 1936; issued February, 1937. Cooperative investigation by the Bureau of Plant Industry, U. S. Department of Agriculture, and the Instituto Biologico de São Paulo. The authors in the beginning undertook these studies independently, and each has made separate contributions on the general subject (*2*, *3*, *21*).[3] The cooperative study of the problem was begun in 1933, and from October 1935 to June 1936 at the invitation of the instituto Biologico, the junior author worked at São Paulo in direct cooperation with the senior author. Since this paper was written the perfect stage of the fungus herein discussed has been found. This discovery led to a change of the title "Sweet Orange Fruit Scab Caused by *Sphaceloma fawcetti viscosa*", originally proposed to the present form. A related paper (JENKINS, A. E., and BITANCOURT, A. A., DOENÇAS DAS PLANTAS POR FUNGOS DOS GENEROS ELSINOË E SPHACELOMA. Rodriguësia. In press.) gives a synopsis of all the known species of *Elsinoë* and *Sphaceloma*.

[2] The authors are indebted to J. M. Toledo, of the Instituto Biologico, for the color drawings shown in pl. 1, *A* and *B*, and to J. Marion Shull, of the U. S. Department of Agriculture, for those in pl. 1, *C* to *L*, and in pl. 8. These plates were furnished by the Instituto Biologico. Many of the photographs were made by members of the Instituto Biologico, namely, J. G. Carneiro, A. Federman, B. U. Mazza, and the senior author.

[3] Reference is made by number (italic) to Literature Cited, p. 16.

[4] In the United States the disease caused by *Elsinoë fawcetti* is called citrus scab, but in this discussion the term "sour orange scab" is used as a matter of convenience to distinguish this disease from those caused by *E. australis* and *S. fawcetti scabiosa*. The choice of the name seems appropriate, since *E. fawcetti* was first observed on the sour orange (*Citrus aurantium* L.), which it attacks virulently.

SPECIES AND VARIETIES OF CITRUS AFFECTED

In São Paulo the sweet orange varieties thus far observed to be affected by the sweet orange fruit scab are the following: Bahia Navel,[5] Pera, Sabará, Selecta, São Sebastião, Santos, Lima, and Mangaratiba. Artificial inoculations show that the Abacaxi variety also is susceptible.

In Tucumán, Argentina, Müller[6] has observed the disease on the following varieties: Ruby Blood, Sweet Mediterranean, Valencia, and Criolla.

Other kinds of citrus known to be affected by the sweet orange fruit scab in São Paulo are the tangerine of Brazil (*Citrus nobilis* var.), tangerona (*Citrus nobilis* × *sinensis)* a sweet lime known as lima da Persia (*C. aurantifolia* Sw.), a sour lime known as limão seda (*C. aurantifolia*), laranja cravo (*Citrus* sp., probably a variety of *C. nobilis* Lour.), and a pointed-leaf papeda (*C. hystrix* DC.). These last two are subject also to sour orange scab. In Argentina, according to G. L. Fawcett (*15*), the fruit scab slightly affects sour orange and mandarin (possibly the same as the variety called tangerine in Brazil).

THE DISEASE

HISTORICAL REVIEW AND GEOGRAPHIC RANGE

Sweet orange fruit scab, as the disease under discussion is designated in this paper, was previously referred to in related papers by the authors as navel orange scab (*21*) and as scab or verrucosis of sweet orange and tangerine (*3*).

In South America sweet orange fruit scab is known to occur in Argentina, Paraguay, Uruguay (apparently), and Brazil. The present knowledge of the distribution of the disease is based on the records detailed below rather than on a planned survey. During the past several years scabbed sweet oranges (including the navel), from Brazil and Argentina, have occasionally been intercepted at seaports of the United States by inspectors of the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.

The earliest record of sweet orange fruit scab is from Paraguay in 1882. This antedates by about 40 years the first records of sour orange scab in South America. These records of sour orange scab are from Argentina (*14*), Paraguay (*36*), and Brazil (*29*).

---

[5] In the present paper, as in a previous one (*21, footnote 2*), the varietal name Bahia Navel refers to the variety of *Citrus sinensis* that is the original source of the Washington Navel orange in the United States. As is also explained in the article just cited, "From plants of the Bahia variety that were introduced into the United States, the late William Saunders, landscape gardener and superintendent of gardens and grounds of the United States Department of Agriculture, budded the 2 trees that were introduced into California in 1873 and from which developed (*13*) what may be termed the 'Washington Selection' of this variety, now known as the Washington Navel." For the botanical classification of the citrus hosts referred to in the article the authors have consulted Swingle (*37*), who is at present engaged in revising and amplifying the treatise cited.

[6] Letter of May 6, 1935, to H. S. Fawcett, Citrus Experiment Station, University of California, Riverside, Calif., from A. S. Müller, Escola Superior de Agricultura e Veterinaria de Minas Geraes, Brazil, descriptive of his trip to Argentina, February 1935.

EXPLANATORY LEGEND FOR PLATE 1

A, Ripe sweet orange affected by the fruit scab (× 1). *B*, Enlargement of lesions (× 8). *C–G*, potato-dextrose agar, parallel cultures of *Elsinoë australis*, *E. fawcetti*, and *S. perseae*: *C*, *E. australis*, convolute type of growth (strain 345); *D* and *E*, *E. fawcetti*: *D*, convolute type (strain 8); *E*, pulvinate type (strain 71): *F* and *G*, *S. perseae*: *F*, convolute type (strain 21); *G*, pulvinate type (strain 363). *H–L*, Same strains on wort agar. (All × 1.) Cultures *C–G*, approximately 3 weeks old; *H–L*, 4 weeks old.

A

B

C

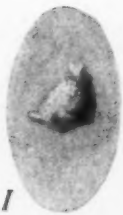
D

E

F

G

H



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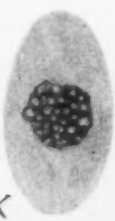

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FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

Comp. Melhoramentos de S. Paulo (Brazil)

In Argentina

G. L. Fawcett, in reporting (1920) the existence of sour orange scab in Tucumán (*14*), stated that the sweet orange is very resistant, although the fruits are sometimes affected. Later (*15*) he noted that the scab of lemon and the scab of sweet orange, as he called the two diseases, differ in regard to their host plants, as well as, apparently, their causal organisms, although in general they are very similar and are controlled by similar methods. More recently he stated[7] that the sweet orange fruit scab was present in Tucumán before the sour orange scab was introduced and that he[8] first observed sweet orange scab there in 1915.

In February and March 1927 Kisliuk[9] reported the finding of scab and scale on oranges from Corrientes, Argentina, in the retail markets of Buenos Aires. A specimen of scabbed fruit collected in February 1927 is in the mycological collections of the Bureau of Plant Industry, and is typical of sweet orange fruit scab. Scabbed sweet orange fruits from Bella Vista, Corrientes, 1934, were contributed by León Grodsinsky, of the Ministerio de Agricultura, Buenos Aires. Marchionatto in 1934 (*27*) reported that in Argentina "citrus scab has been observed extensively during the present year on various citrus fruits (oranges, mandarins, and grapefruit) in citrus growing districts of the coast." In part the disease referred to was probably sweet orange fruit scab. In the letter by Müller[10] already cited he stated that he had spent the month of February traveling through widely separated parts of Argentina (including Tucumán), and that what impressed him most in citrus diseases was the abundance of sweet orange fruit scab; wherever he saw orange trees these were affected by scab, whereas the sour orange scab was comparatively rare.

In Paraguay and Uruguay

In Paraguay and Uruguay, in 1920, Spegazzini (*36*), describing symptoms, evidently those of sour orange scab, on leaves of lime (*Citrus aurantifolia*), stated that growers there had informed him that the disease also attacks shaddocks, sweet oranges, and lemons, but that he had not had an opportunity to confirm these statements at first hand. There is, of course, the probability that the disease of sweet orange was the sweet orange fruit scab. On several occasions mention has been made (*20*, *21*, *26*) of the mycological specimen showing what are now considered to be typical lesions of sweet orange fruit scab, collected at Villa Rica, Paraguay, in 1882 by Balansa[11] (B. Balansa Pl. du Paraguay no. 3543).

The scab of sweet orange which Girardi (*17*) reported in 1922 as having been present in Paraguay in a mild form for 30 years is possibly the sweet orange fruit scab. Girardi stated that the small isolated or grouped lesions of the disease had been confused by some with certain scale insects.

---

[7] Letter to H. S. Fawcett, dated Dec. 20, 1931.
[8] Letter to H. S. Fawcett, dated Nov. 17, 1933.
[9] Kisliuk, M., Jr. Report of fruit fly survey—Argentina. U. S. Dept. Agr., Bur. Ent. and Plant Quarantine. pp. 4–5, 20. 1927. (Unpublished.)
[10] See footnote 6.
[11] As recently noted by Vellard (*38*), Balansa, whose grandsons are living in the region of Villa Rica, introduced into Paraguay the distillation of petitgrain oil, which is obtained from sour orange (*Citrus aurantium*).

In Uruguay, with respect apparently to sweet orange fruit scab, Girardi reported further that the disease was alarmingly prevalent, there being no orange groves entirely free from it. He added that if the disease has not been given the importance it deserves it is because growers have believed it to be due to adverse conditions of locality or weather.

In Brazil

In Brazil sweet orange fruit scab is known to occur in the States of São Paulo, where are grown about 60 percent of the Brazilian oranges that are exported, Rio Grande do Sul, Minas Geraes, Rio de Janeiro

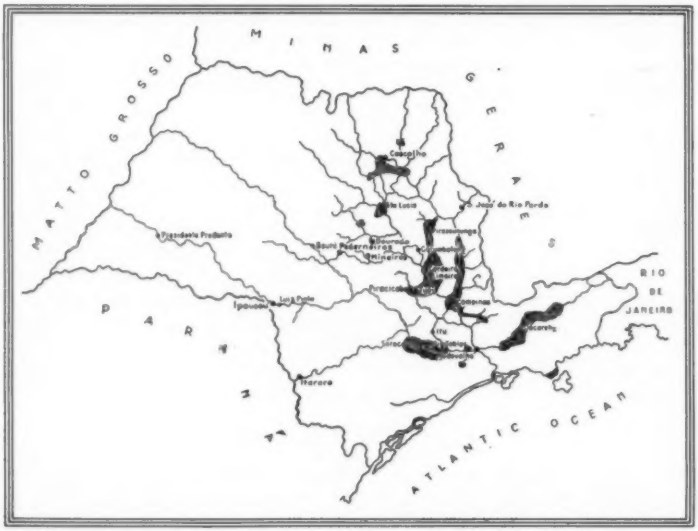


FIGURE 1.—Map of the State of São Paulo, Brazil, showing location of neighboring States. Shaded areas indicate the orange-growing districts of São Paulo and names of places show localities where sweet orange fruit scab is definitely known to occur. Recent distribution not indicated on the map includes the following localities: Caraguatatuba, Cravinhos, and Torrinha (apparently).

and in the Districto Federal. Unverified reports indicate that it may also be present in the States of Matto Grosso and Paraná (fig. 1).[12]

In the light of present knowledge it is believed that Noack's (*31*) description of scab (Grind) on fruits and young twigs of various kinds of oranges (an verschieden Orangensorten) in São Paulo (1900) may have been sweet orange fruit scab.

In São Paulo sweet orange fruit scab came definitely to the attention of citrus growers about 1931, and since that time it has come to be regarded as an important disease (*2, 3, 9, 21, 41*). At first it was observed only in a few localities, but, by 1934, it had been found to occur abundantly in the most important commercial citrus-growing districts (*41*). Carvalho (*9*) stated in 1932 that severely diseased

[12] It was previously assumed (*21*), from Bondar's (*7*) account of citrus scab in Brazil, that in the State of Bahia the sweet orange is affected by scab. More recent inquiries, however, indicate that this interpretation may not be correct. Sweet orange fruit scab had not been distinguished from sour orange scab when Bondar's paper was written, and in a personal conference with the authors on Oct. 29, 1935, Bondar expressed uncertainty as to whether the sweet orange fruit scab is found in Bahia. O. H. Leonardos, of the Estação Experimental de Citricultura, Alagoinhas, Bahia, on Aug. 19, 1935, wrote that as yet the sweet orange fruit scab is not known in Bahia.

sweet oranges of various varieties had been received from Limeira, Sorocaba, and Piracicaba. The first critical studies of the disease which demonstrated it to be separable from sour orange scab are those of the present authors. (See footnote 1.)

Authority for the occurrence of the disease in Rio Grande do Sul is based upon a herbarium specimen collected near Borges de Medeiros Instituto, Porto Alegre, in March 1923, by E. Bruck. The specimen (no. 1745, Secção de Phytopathologia do Instituto de Biologia Vegetal, Rio de Janeiro) was later identified by E. Rangel as *Sphaceloma fawcetti* Jenkins, as the sour orange scab fungus was designated in 1925 (*19*). A note on the label of the specimen states that the characters observed are those of the pseudocanker of Costa Rica. According to Smith (*35, p. 62 and legends to figs. 45 and 46*), the pseudocanker of Costa Rica is the same as sour orange scab. The following statement, translated from the Portuguese (*34*), is indicative of the prevalence of the sweet orange fruit scab in 1936 in the vicinity of Porto Alegre:

In our almost daily inspection of the packing houses of this region we find that a great majority of the fruits unsuitable for exportation is rejected owing to the presence of "spots" caused by the fungus disease known by the common name of verrucosis or scab. A large percentage of the fruits rejected in these packing houses is made up of oranges showing this disease.

The record of the occurrence of the disease in Minas Geraes is based on a few fruits of the Bahia Navel from Jacutinga, collected on August 8, 1934 (*28*), and deposited in the mycological herbarium of the Escola Superior de Agricultura e Medicina Veterinaria de Minas Geraes. A specimen from this collection was observed by the authors in January 1936.

In the Districto Federal, at Campo Grande, the disease was recently (1935) observed on the Pera and Selecta sweet oranges in two or three orchards. It has since been found (1936) in numerous other places not only in Campo Grande, but also in Nova Iguassú and São Gonçalo in the State of Rio de Janeiro. According to quarantine officers there are now more than 60 groves in this general region where the disease has been identified. In a grove at Inhoahyba, Districto Federal, there is an abundance of sweet orange fruit scab. On a tree of the Selecta variety leaves as well as fruits were heavily scabbed.

ECONOMIC IMPORTANCE

Sweet orange fruit scab apparently does not affect the general health of the sweet orange. Severely diseased fruits often reach normal size, but the rind is greatly disfigured. There is apparently no loss in the flavor of the fruit. This partly explains why citrus growers in Brazil paid little attention to scab while their oranges were produced for local consumption or for exportation on a small scale to Argentina and Uruguay. Upon the exportation of oranges to Europe (beginning in about 1929), however, a series of Federal regulations was inaugurated in order to prevent the shipment of low-grade fruit. The fruit scab then began to be a matter of serious concern to citrus growers in São Paulo. In some groves from 50 to 60 percent of the fruit was blemished by the disease, some of it

severely, and in numerous other groves as much as one-third of the crop was unfit for export (*41*). Fortunately, however, as shown experimentally, the disease can be controlled (*41*), and this has since been demonstrated under commercial conditions by the results of spraying in a number of orchards.

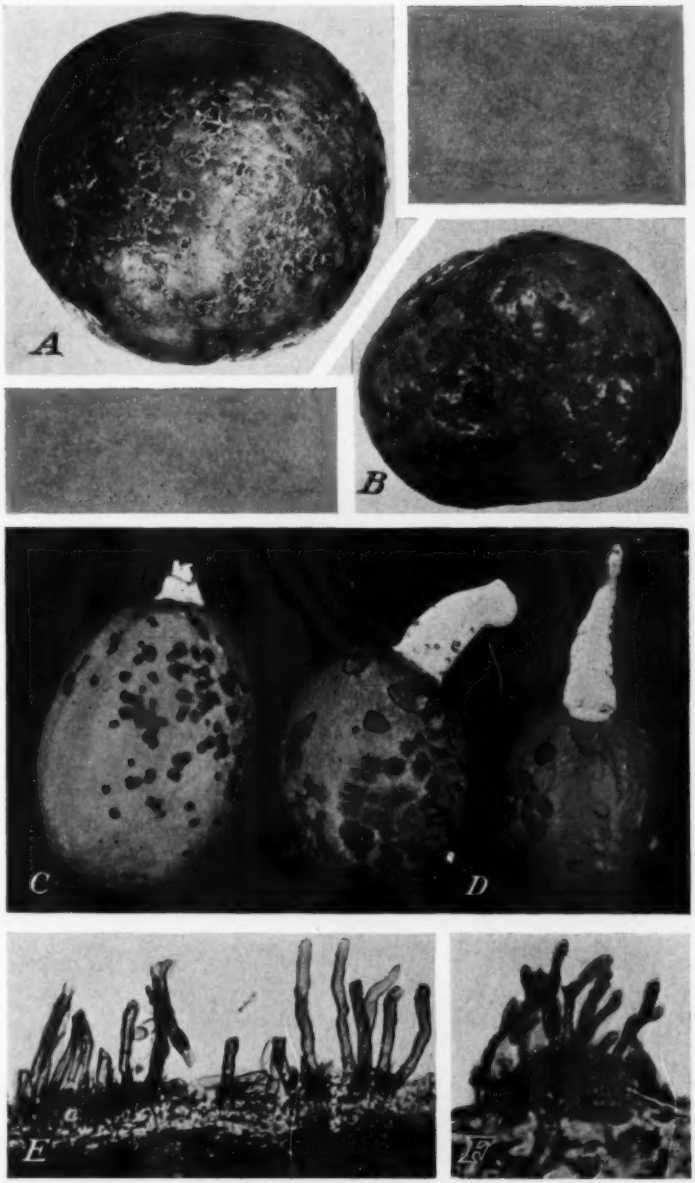
SYMPTOMATOLOGY

Morphologic Symptoms

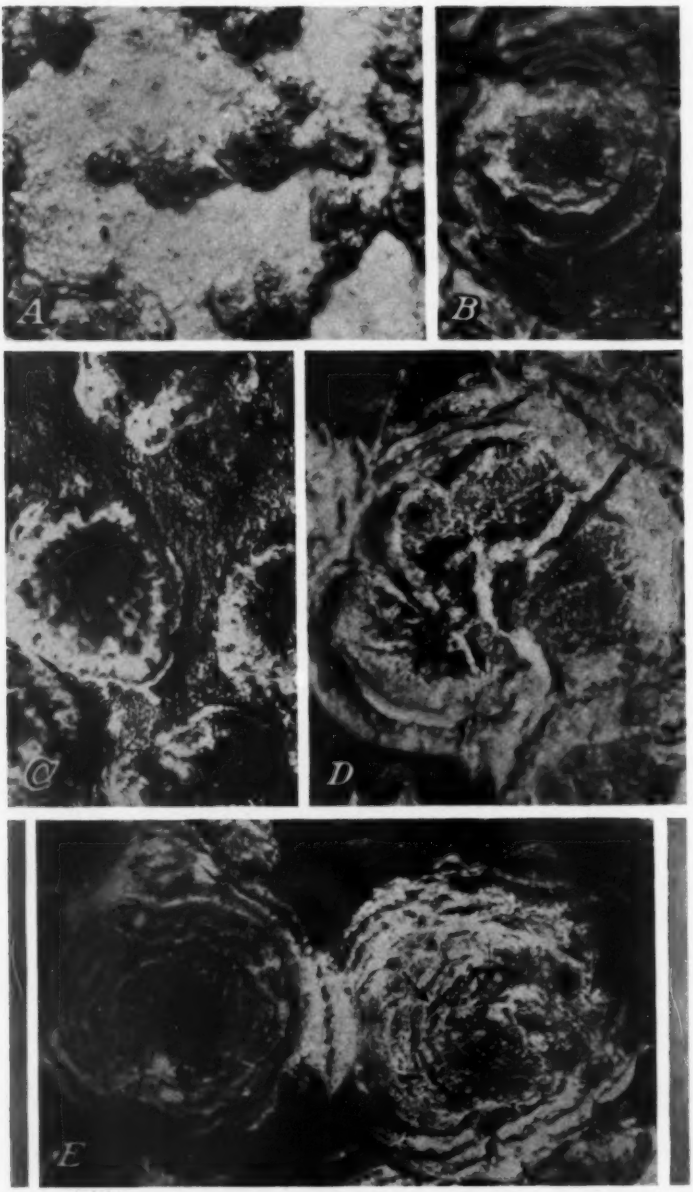
The symptoms of sweet orange fruit scab detailed below are more or less supplementary to those previously given by one of the authors (*2, 3*). So far as observed in South America, sweet orange fruit scab affects mostly the fruits, while sour orange scab attacks leaves and young twigs as well. Lesions on leaves of sweet orange are so rare that it has been the belief for some time that the leaves are entirely free from the disease, and this opinion was expressed by G. L. Fawcett (*15*) and by Honey (cited in *21*). As a result of a very thorough examination of a navel orange grove in Limeira, however, during one of the most severe attacks of the disease yet noted, a number of mildly scabbed leaves were found in the inner part of the tree. In general, leaves of the outer part of the crown, and, in fact, more than 90 percent of the leaves of severely diseased trees, do not show lesions at all. Although from the standpoint of symptoms the leaves may be considered as generally scab-free, leaf infection is not to be disregarded, for doubtless even a few lesions may play an important role as hold-over scabs.

On Fruits

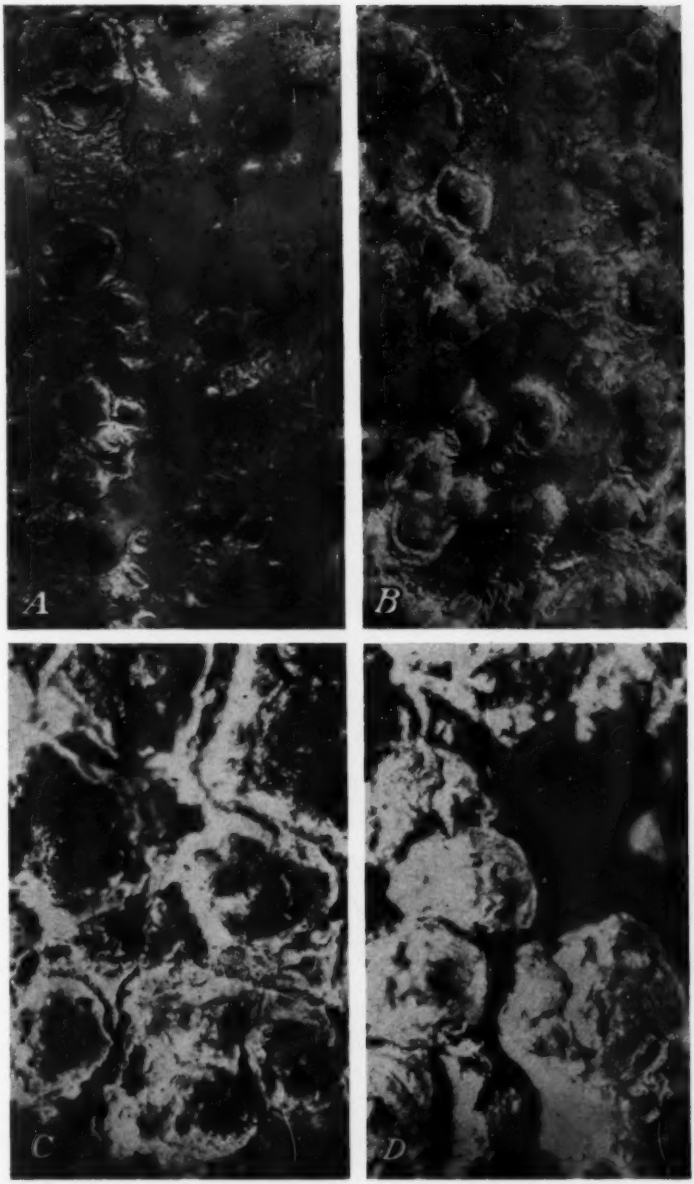
Lesions of sweet orange fruit scab on young fruits may cause an appreciable deformation of the rind, which is usually protuberant or swollen where the lesions appear. These deformations gradually disappear as the fruit increases in diameter, and mature fruit has the normal spherical shape. The surface, however, is sometimes slightly depressed at the points where lesions are large and coalescent (see pl. 12, *C*). In general appearance the lesions (pl. 1, *A* and *B*; pl. 2, *A* and *B*) are similar to those of sour orange scab. Upon careful examination, however, it is possible to discern certain appreciable differences to be explained presently. Individual lesions of the sweet orange fruit scab consist of pustules of corky tissue, which are round to irregular, raised, and usually slightly convex (pl. 3, *A*) measuring from 2 to 6 mm in diameter. The larger lesions (pl. 3, *B–D*) are more flattened and may be traversed by more or less deep furrows which result from the rupture of the corky tissues. In some cases a circular fissure (pl. 3, *B*) surrounds the central part of the lesion and this part may be detached with comparative ease, leaving in its place a slightly depressed scar covered with a silvery pellicle. This same pellicle sometimes surrounds more extensive lesions or covers the areas between them to a greater or less extent (pl. 4, *A*). On severely infected fruits the lesions are frequently large and confluent and cover extensive areas (*2, fig. 44*), rendering the fruit too unsightly for export, as previously mentioned. In some cases the smaller and more numerous lesions resulting from secondary infection may be distinguished from the primary lesions, which they surround on the same fruit (*2, fig. 47*).



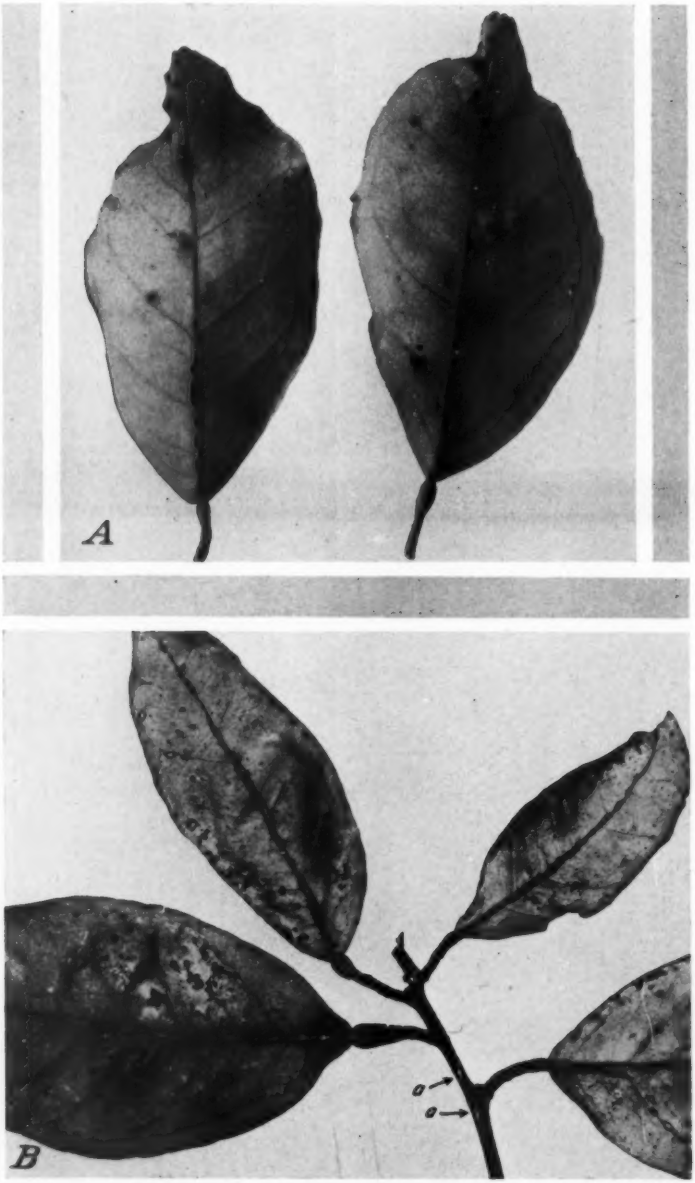
A and *B*, Lesions of sweet orange fruit scab on tangerine from a market in São Paulo (× 1). *C* and *D*, Young avocado fruits of the Guatemalan race from Limeira, São Paulo, affected by avocado scab: *C*, Linda variety; *D*, Winslow variety (× 1). *E*, Conidiophores of *Sphaceloma perseae* from avocado leaf (× 450). *F*, Conidiophores of *E. fawcetti* from a lemon leaf (× 480).



Enlargements of scab lesions of sweet orange fruit scab on (*A*, *C*, and *D*) sweet orange and on (*B* and *E*) tangerine (all × 17); *B*, *a* and *E*, *a*, pustules of the causal fungus.



Lesions of sweet orange scab on (*A*) sweet orange, and lesions of sour orange scab on (*B*) lemon and (*C* and *D*) sour orange. *A* and *B*, × 7½; *C* and *D*, × 17.



A and *B*, Lesions of sweet orange fruit scab on leaves and twig (*B*, *a*) of sweet orange. × 1.

The coloration of the lesions of sweet orange fruit scab is determined on the one hand by the color of the corky tissues and on the other by the conidial pustules of the parasitic fungus. The corky tissues are "light ochraceous buff" [13] to "warm buff." Sometimes there is superimposed on these tissues a reddish tinge, "deep vinaceous" to "eugenia red", which is also noted in the rind surrounding the lesions. The conidial pustules of the causal fungus of the disease, often visible near the center of the lesion, are "warm buff" to "clove brown", and "fuscous-black." In general the lesions, together with the exposed growth of the pathogen, thus exhibit a variety of colors, ranging from buff to black with sometimes a pinkish hue, which can be distinguished without the aid of a hand lens. The dark-colored lesions may resemble lesions of scab of apple (*Malus sylvestris* Mill.) caused by *Venturia inaequalis* (Cke.) Aderh., as noted by Carvalho (*9*).

Comparing the lesions of sweet orange fruit scab on sweet orange (pl. 4, *A*) and tangerine with those of sour orange scab on lemon (*B*) or sour orange (*C* and *D*), it will be seen that the former, as a rule, are more nearly circular, and, particularly on the tangerine (pl. 3, *E*), more flattened and less wrinkled or rough. Fruit lesions of sour orange scab often consist of irregular pustules, on the surfaces of which appear small rounded elevations or wrinkled protuberances (pl. 4, *C* and *D*) not found in sweet orange fruit scab. The corticose tissues in the fruit lesions caused by sour orange scab also seem to be more spongy and less compact than in those caused by sweet orange fruit scab. It was evidently sour orange scab lesions of the type shown in *D*, although much more pronounced, that Spegazzini (*36*) described as "nodules having a granular surface almost like a cauliflower."

Reference to lesions of both sweet orange fruit scab and sour orange scab on a common host, i. e., fruits of laranja cravo, is made in the section entitled "Inoculations."

In plate 2, *C* and *D*, is shown an example of avocado scab, which, as already indicated, is similar to sour orange scab but distinct from both this disease and sweet orange fruit scab.

Symptomatological characters of the scab disease of the remote citrus relative *Hesperethusa crenulata* (Roxb.) Roemer have a certain similarity to those of sweet orange fruit scab (*24*).

ON LEAVES AND TWIGS

On the leaves of sweet orange the lesions are mostly found only on the lower surface, chiefly on the midrib (pl. 5, *A* and *B*). They seldom form protuberant outgrowths as on the fruits, although in very young leaves they sometimes form funnellike pockets (*A*) similar to those produced in sour orange scab. The leaf lesions are smooth, and frequently have a somewhat glossy surface. Their color agrees with that of the fruit lesions, except that they may be bordered by a narrow brown line. The lesions, with the exception of those along the midrib, seldom attain 2 mm in diameter. On pointed-leaf papeda and Selecta orange the leaf lesions may be distributed on both leaf surfaces, as is the case with sour orange scab. In general, as thus far observed, the leaf lesions of sweet orange fruit scab are smaller and less elevated than those of sour orange scab.

[13] The color readings given in quotations are based on Ridgway's Color Standards (*33*).

On the twigs (pl. 5, *B*, *a*) the lesions of sweet orange fruit scab are also comparatively rare and inconspicuous, resembling those on the midrib of the leaves.

Signs

On fruits, on the surface of the lesions as well as within the fissures, there may be seen with the aid of a powerful lens small buff to black projections or pustules (pl. 3, *B*, *a*), the largest of which are about 0.1 mm in diameter. They are sometimes concentrically arranged and more or less continuous (*E*, *a*), or they may be aggregated at the center of the lesion (*B*, *a*) or more or less effuse over the entire central part of the scab (*C*). The projections are the conidial pustules or acervuli and sporodochia of the causal fungus of the fruit scab, referred to on page 7. Signs on leaves are generally inconspicuous, although on pointed-leaf papeda they may be prominent.

The concentric arrangement of fructifications in the case of sour orange scab (*20, fig. 2, E*) is less evident than in sweet orange fruit scab. The outstanding sign of sour orange scab is of course the Cladosporium-like development of the fungus on the surface of fairly young lesions, and this type of growth has not been observed for the causal fungus of sweet orange fruit scab.

Histologic Symptoms

The hyperplastic nature of the lesions of sweet orange fruit scab (pl. 6, *A*) is generally similar to that of sour orange scab as described by Cunningham (*11*) and as later discussed by Butler (*8, p. 181*).

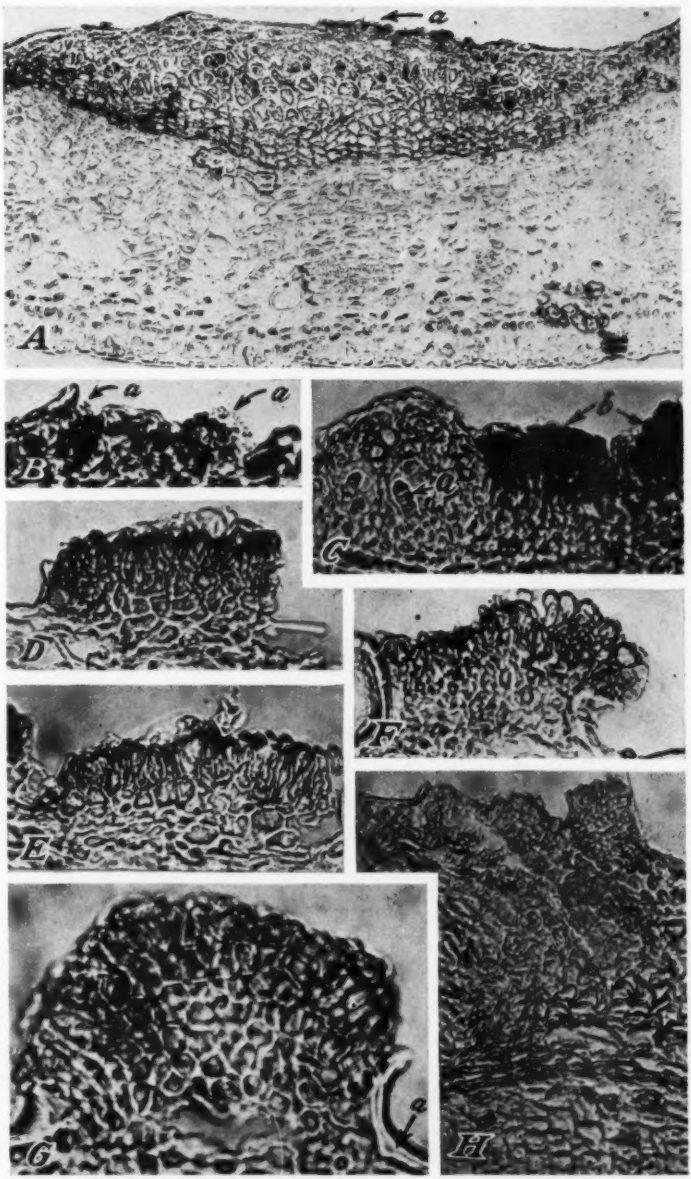
THE CAUSAL FUNGUS

NAME, HISTORY, AND CLASSIFICATION

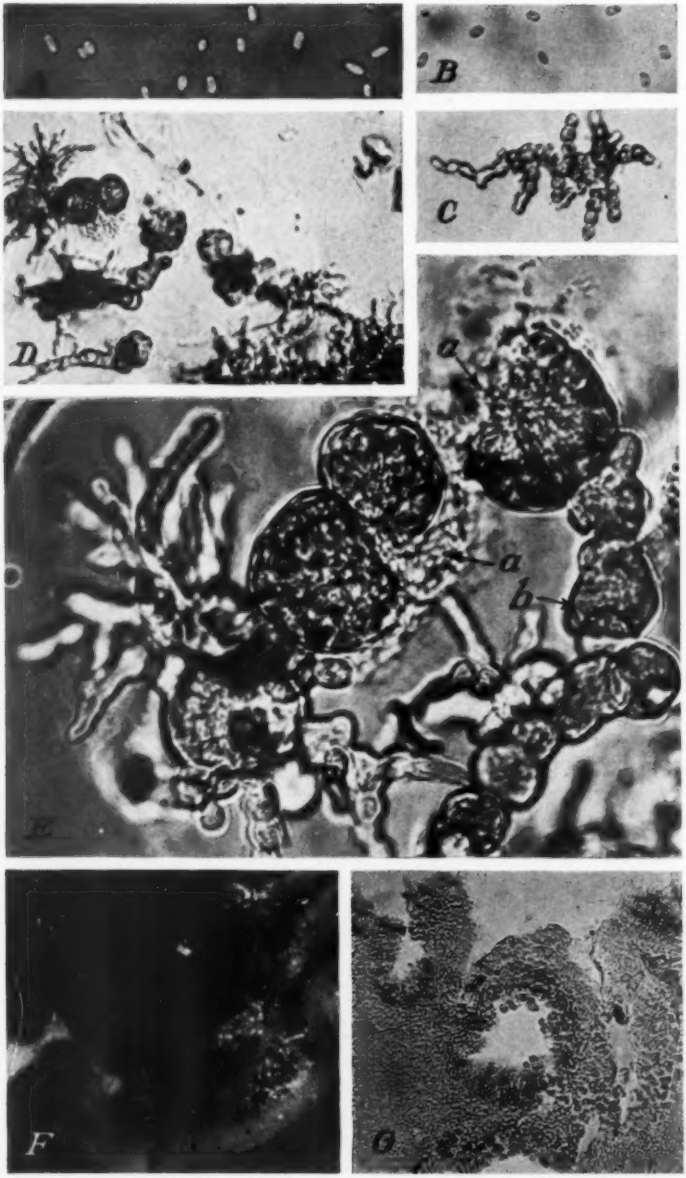
The fungus causing sweet orange fruit scab is the myriangiaceous ascomycete *Elsinoë australis* Bitancourt and Jenkins (*5*), originally described (1933) as *Sphaceloma fawcetti viscosa* Jenkins (*21*). The earliest available record of the fungus is the previously mentioned specimen on rind of sweet orange collected in Paraguay in 1882. The general appearance of this specimen, together with the original label, is shown elsewhere (*26, fig. 1, A and B*); in plate 6, *H*, are shown acervuli from this material. The perfect stage of the fungus was discovered in July 1936, on scab lesions on a ripe Bahia Navel orange (*5*). In describing this stage as a distinct species it was suggested that the imperfect stage be called *Sphaceloma australis* Bitancourt and Jenkins. In its perfect stage the fungus differs from *Elsinoë fawcetti* in the apparent absence of an epithecium and in having well-developed globose ascomata and longer ascospores. The conidial stage is not characterized by the Cladosporiumlike development of *E. fawcetti* and is more persistent.

MORPHOLOGY

The conidial fructifications of *Elsinoë australis* consist of acervuli and sporodochia (pl. 6, *D–H*) arising from intraepidermal or subepidermal hyphae. Conidia other than the bodies interpreted as microconidia have been uncommon and usually entirely absent on these fructifications as collected in the field and examined in the laboratory. The bodies interpreted as microconidia are usually present on the surface of the acervuli and sporodochia (pl. 6, *B*, *a*). They



A, Section of scab lesion on sweet orange leaf; pustules of causal fungus, *Elsinoë australis*, visible at *a*. × 150. *B*, Enlargement of *A*, *a*, showing ruptured epidermis and masses of microconidia (*a*). × 800. *C*, Ascoma; *a*, 3-septate ascospore in ascus; *b*, conidial layer. × 500 (reproduced from (4)). *D–H*, Conidial fructifications of the fungus on rind of (*D–E*) tangerine and (*F–H*) sweet orange. *G*, *a*, outer wall of ruptured epidermis. *D–F*, × 650. *G*, × 870; *H*, section of specimen from Paraguay, collected by Balansa in 1882. × 200.



A and *B*, Conidia of *Elsinoë australis* (*A*) and of *E. fawcetti* (*B*) from culture. × 380. *C–G*, *E. australis*: *C*, germinated conidium on malt agar (× 290); *D*, greatly swollen germinated conidia on potato-dextrose agar, the larger cells filled with microconidia (× 220); *E*, enlargement of *D*, showing conidia (*a*) escaping from ruptured cells and younger hyphal growth (*b*) containing microconidia (× 700); *F*, pycnidialike development in culture (× 5); *G*, section of these structures (× 150).

are hyaline, often refractive, and bacteriumlike in appearance and they stain readily with cotton blue.

In culture the previously reported (*21*) ovoid to oblong-elliptical hyaline conidia of *Elsinoë australis* may be present on the surface of fairly old growth. Those here illustrated, both of *E. australis* (pl. 7, *A*) and of *E. fawcetti* (*B*), were produced from small masses of potato-dextrose agar cultures transplanted to a film of malt agar on a glass slide, the culture having been held for 24 hours in a Petri dish lined with wet filter paper. Numerous germinated conidia or young thalli (*C*) were present in the culture of *E. australis* when it was several days old. In similar potato-dextrose agar cultures, as previously noted (*21, footnote 11*), the cells of greatly swollen germinated conidia (*D*) were filled with microconidia measuring 1μ to 3μ by 0.5μ (*E, a* and *b*). In one instance black pycnidialike masses were produced by the fungus grown on potato-dextrose agar slants (pl. 7, *F* and *G*).

The perfect stage of the fungus (pl. 6, *C*) is described (*5*) as follows:

> Ascomata globose, sometimes flattened or irregular, occasionally confluent, 40—120 μ in diam., buff, embedded in the tissues of the perfect stage, erumpent, consisting of a hyaline or slightly yellowish pseudoparenchyma devoid of a well defined epithecium; asci often distributed in the upper part of the ascoma, globose to obclavate, inner wall thickened apically, 15—27×13—21 μ; ascospores hyaline, variable, straight or more or less curved, 2—4 celled, often markedly constricted not only at median septum, but also at the other two [septa], sometimes with a longitudinal septum in the upper middle cell, which is frequently slightly larger than the other cells, 12—20×4—8 μ.

ISOLATIONS AND CULTURAL COMPARISONS

As previously reported (*21*), to assist in the identification of *Elsinoë australis* as found on the Bahia Navel orange, two different isolations were first made, one from the specimen from Sorocaba (strain 345) and one from the specimen from Limeira (strain 356). The two strains were similar in appearance but were separable from the other strains of *E. fawcetti* then at hand. They were also culturally distinct from *S. fawcetti scabiosa*, then not distinguished from the species. The cultures of *E. australis* were particularly distinctive in that they were copiously covered with a clear viscous substance (*21, fig. 1, C and D*).

The cultural growth of *Elsinoë australis* was of the convolute type, and was therefore directly comparable to the convolute growth types of *E. fawcetti* and *S. perseae* rather than to their pulvinate growth types.[14] This is brought out in plate 1, *C* to *G* and *H* to *L*, which also shows *E. australis* to be culturally distinct from *E. fawcetti* and *S. perseae* on both potato-dextrose and wort-agar media.

To obtain further data on the identity of the organism associated with sweet orange fruit scab, additional isolations of this variety and of *E. fawcetti* and *S. perseae* were made from leaves and fruits of their respective suscepts (table 1), and then additional parallel cultural comparisons were made.

Various methods were employed in making the isolations, but for the most part they were made from scrapings of lesions. These were placed in a Petri dish, and the contents of a culture tube containing potato-dextrose or glycerin agar was poured over them. For some of the isolations of *Elsinoë australis* (for example, culture 702A) sections of conidial fructifications similar to those shown in plate 6, *D* to *F*,

[14] The so-called convolute and pulvinate types of growth as produced by *S. fawcetti* and *S. perseae* have been described in related papers (*20, 22, 25*).

were transplanted to the edge of agar slants. The development of the fungus could be observed from the first under a comparatively high magnification. For another isolation a water suspension of conidia was made on a glass slide, allowed to dry, and then a single conidium was transferred to the edge of the slant with a micro-needle.

TABLE 1.—*Isolations of Elsinoë australis and E. fawcetti employed in a parallel cultural comparison on potato-dextrose agar (pl. 9, A–D)*

Arrangement in plate 9, *A–D*	Strain no.[1]	Organism	Source of material from which isolations were made		
			Host plant		Country
			Scientific name	Common name or variety	
A and *B*:					
a	345a	*E. australis*	*Citrus sinensis*	Bahia Navel	Brazil.
b	345	do	do	do	Do.
c	381B	do	do	do	Do.
d	356	do	do	do	Do.
e	375	do	do	Sweet orange	Argentina.
f	379	do	do	do	Brazil.
g	933A	do	*C. nobilis deliciosa*	Tangerine	Do.
h	702A	do	*C. sinensis*	Sweet orange	Do.
C and *D*:					
a	8	*E. fawcetti*	*C. limonia*	Rough lemon	United States.
b	275	do	do	do	Union of South Africa.
c	376	do	do	do	Brazil.
d	828A	do	*C. aurantium*	Sour orange	Do.
e	259	do	*C. mitis*	Calamondin	Japan.
f	826A	do	*C. grandis*	Grapefruit	Brazil.
g	776A	do	*C. limonia*	Sicilian lemon	Do.
h	806A	do	do	Sweet lemon	Do.
i	745A	do	*Citrus* sp.	Rangpur lime	Do.
j	823A	do	*C. aurantifolia*	Tahiti lime	Do.
k	121B	do	*C. nobilis*	Laranja cravo	Do.

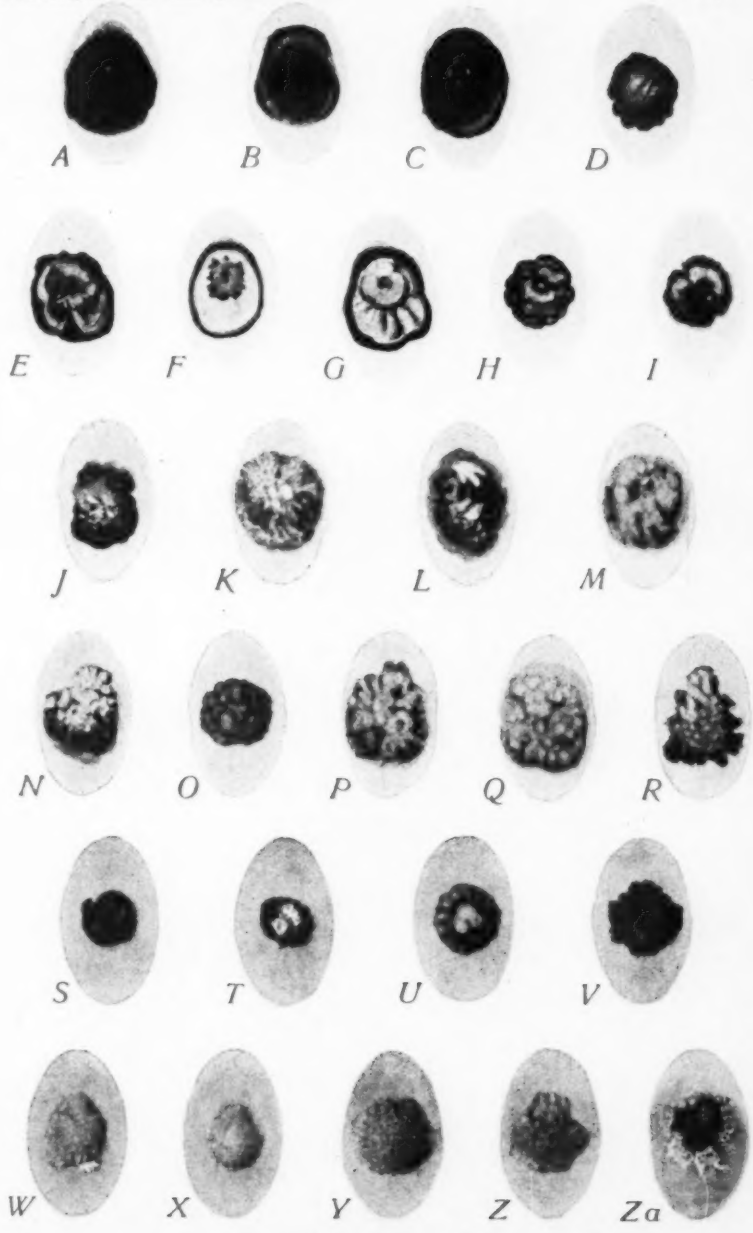
[1] Strain numbers followed by a capital letter are those of the Secção de Phytopathologia do Instituto Biologico de São Paulo, and represent isolations made by the senior author; those not followed by a letter represent other isolations, in the collection of the junior author.

The cultural comparisons shown in plate 8 were made in 1933, when there were available three new isolations of the *Sphaceloma* from citrus scab from South America. Two were of *Elsinoë australis* from common sweet orange (strains 375 and 379) and one of *E. fawcetti* (*21, footnote 11*). These strains (grown on tubes of the size shown in plate 9, *A–D*) are here compared with one of the original strains of *E. australis*, and with *E. fawcetti* and *S. fawcetti scabiosa*. On the three different substrates, malt agar (pl. 8, *A–I*), potato-dextrose agar (*J–R*), and wort agar (*S–Za*), the new strains from sweet orange formed a group with the original strain of *E. australis*, although all three could be distinguished from one another. Strain 379 on potato-dextrose agar (*K*) was more or less intermediate between *E. australis* and *E. fawcetti*. In another comparison of the three strains on potato-dextrose agar they were again separable when grown at room temperature, but similar when grown in a refrigerator (pl. 11, *D–I*).

A parallel cultural comparison, made on March 20, 1934, included representative isolations of all the strains of *Elsinoë australis* (pl. 9,

EXPLANATORY LEGEND FOR PLATE 8

A–I, Month-old cultures on malt-agar test-tube slants, of strains 375 (*A*), 379 (*B*), and 356 (*C*) of *Elsinoë australis* and of strain 376 (*D*) of *E. fawcetti* from São Paulo as compared with strains 8 (*E*) and 71 (*F*) of *E. fawcetti* from Florida, strain 200 (*G*) of *Sphaceloma fawcetti scabiosa* from Australia, strain 259 (*H*) of *E. fawcetti* from Japan, and strain 275 (*I*) of the *Sphaceloma* on citrus from the Union of South Africa. *J–R*, Same strains on potato-dextrose agar. *S—Za*, Same strains on wort agar. (All × 1.)



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

Comp. Melhoramentos de S. Paulo (Brazil)

A and *B*) and of *E. fawcetti* (*C* and *D*) then available (table 1). Several strains of *S. perseae* were added to the comparison, but they were readily distinguishable as not of the *E. fawcetti* group and are not reported upon further. The pulvinate strain (345a) of *E. australis* (pl. 9, *A*, *a*, and *B*, *a*) was isolated from strain 345 (*A*, *b* and *B*, *b*). A pulvinate strain (828A) of *E. fawcetti* is shown in plate 9, *C*, *d*, and *D*, *d*. The other strains included in the comparison were of the convolute type of growth. The cultures of *E. fawcetti* were remarkably uniform in appearance (pl. 9, *C*, *a–c*, *e–k* and *D*, *a–c*, *e–k*). They were a bright clear color and generally "vinaceous" to "pinkish vinaceous." In corresponding cultures of *E. australis* the light-colored regions were generally "cinnamon buff", "cinnamon", or "clay color." Strain 375 (*A*, *e*, and *B*, *e*) was, as usual, nearly all black; strain 379 (*A*, *f*, and *B*, *f*) was "pinkish vinaceous."

SALTATIONS

The comparisons just described showed that the strains of *Elsinoë australis* could be divided into two main groups: Group 1, represented by the first two isolations, strains 345 and 356, and by 381B (pl. 9, *A*, *b–d*); and group 2, represented by strains 375, 379, 933A, and 702A (*A*, *e–h*). The existence of these groups was further emphasized by a number of new parallel series with additional new isolations of *E. australis*. These series have also shown that the fungus is so extremely variable that within the two groups many of the isolates may be distinguished from each other. Furthermore, it was soon recognized that within a single isolate there occur variations of the type known as saltations. These are conspicuous in large thalli, and Kolle flasks are convenient for the cultural work with them.

Examples of groups 1 and 2 and of saltations produced from each are here given as representative of variations of *Elsinoë australis* observed on potato-dextrose agar in Kolle flasks.

Strain 381B, representing group 1, is shown in plate 10, *A*. This culture is here distinguished chiefly by the copious mucous covering already mentioned and by the light color of the thallus, "cinnamon buff" to "clay color." There are sometimes embedded in the mucous covering a number of globose bodies (pl. 10, *A*, *a*) of a darker color than the thallus, "sayal brown" to "mikado brown." These are interpreted as secondary thalli probably produced from conidia or detached cells of the primary thallus. In corresponding slant cultures held in an upright position the inoculum for secondary colonies would be carried with the slime to the previously sterile agar surface and there would develop into characteristic thalli. Such secondary growth, produced by strain 375, of group 2, on old cultures of wort and potato-dextrose agar is shown in plate 9, *E* and *F*, respectively, and also by *Elsinoë fawcetti* on potato-dextrose agar in plate 9, *G*.

A saltation isolated from a sector of 381B is characterized by absence of viscosity (pl. 10, *B* and *C*). It is slightly convolute in the central part, and the marginal region is applanate with well-marked concentric zones. The surface is covered with a short white down. The general color is the same as in normal sectors or slightly darker ("light drab") along the radial and concentric zones.

Strain 702A, representing group 2, is convolute and coal black. There are no traces of mucus, and tufts of white down are present on

the surface (pl. 10, *D*, *a*). From this culture has arisen a fan-shaped sector (*D*, *b*) of the pulvinate type of growth. It is concentrically zonate and uniformly covered with a short red down, "Hay's russet" to "dragon's-blood red." A bright-red color, "carmine" and "ox-blood red", is imparted to the medium. A similar, but less pronounced, coloration is produced by the saltation described for group 1.

A further description of variations of this species is published elsewhere (*6*). More or less similar variations may be cited for other organisms, as, for example, those reported by Conant (*10*), Davis (*12*), Hansen (*18*), and Muskatblit (*30*).

In the case of *Elsinoë veneta* (Speg.) Jenkins the decided difference noted between strains isolated by Anderson and Kadow (*1*) and a strain isolated by L. K. Jones [15] is explainable on the basis of what has been referred to here as the convolute and pulvinate types of growth. The strain isolated by Jones was pulvinate in character when first studied by Jenkins (*25*, pl. 1) and it has so remained. Anderson and Kadow (*1*) stated that this strain produced no conidia on corn-meal agar, while the other strains isolated by them produced conidia in abundance. This culture produced some conidia when studied in 1924–25 by Jenkins, but abundant conidial formation was not obtained.

TEMPERATURE RELATIONS IN CULTURE

To obtain data on the temperature relations of *Elsinoë australis*, cultures 381B and 702A, representing groups 1 and 2, respectively, were grown on potato-dextrose, glycerin, and rice agar slants. The cultures were held for 1 month at the following temperatures: 5.5°, 12°, 17.5°, 22°, 26.5°, 31°, 35°, and 39.5° C. The reaction to temperature was the same in all three media. There was no growth at 5.5° and 39.5°, some growth at 12° and 35°, and good growth at 31°. The best growth occurred at 26.5°.

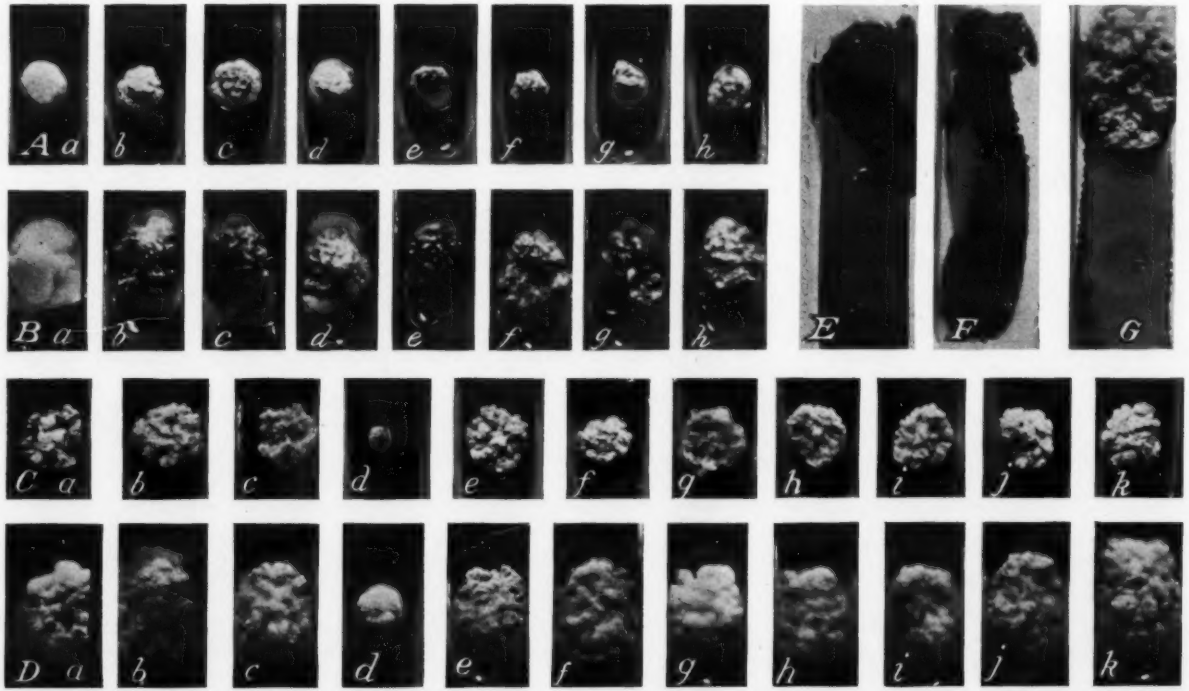
TABLE 2.—*Average diameter of potato-dextrose agar slant cultures of Elsinoë australis and E. fawcetti held for 1 month at different constant temperatures*

Organism	Strain no.	Diameter at indicated temperature							
		3.5° C.	9.5° C.	15° C.	20° C.	24.5° C.	29° C.	32.5° C.	37° C.
		Mm	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*
E. australis	702A	[1] 1	5	8	9	15	14	9	[1] 1
E. australis	381B	[1] 1	5	8	13	19	17	11	[1] 1
E. fawcetti	776A	[1] 1	6	11	14	13	7	2	[1] 1

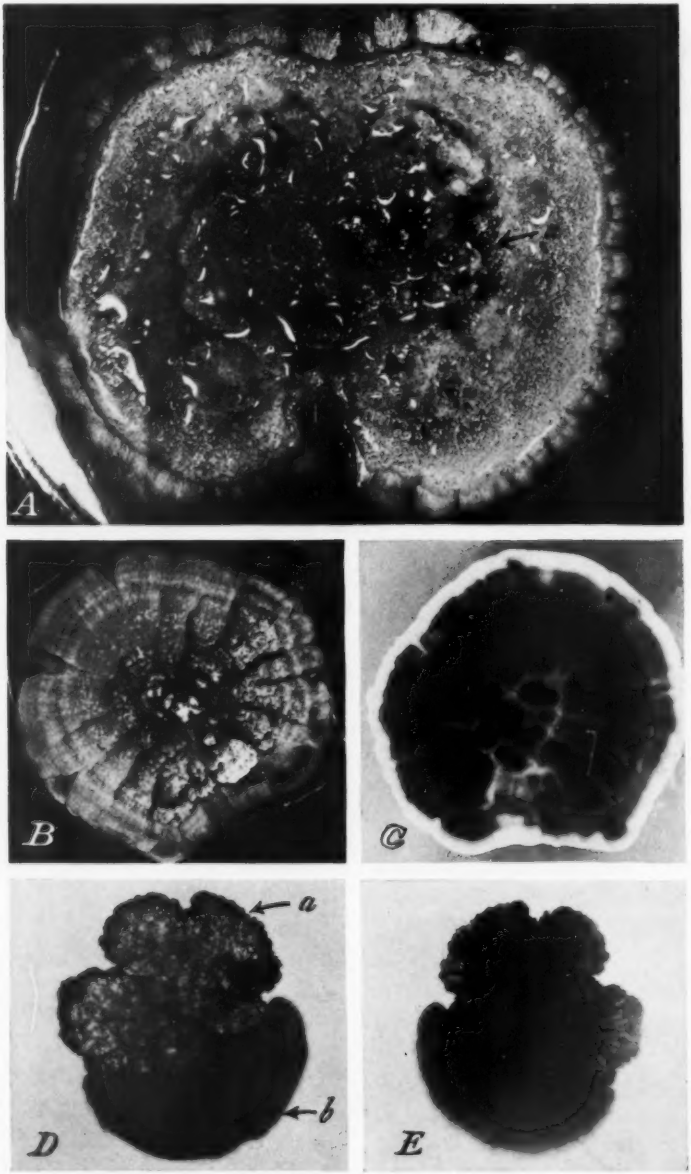
[1] Approximate diameter of original planting.

Following this preliminary experiment, a similar test was made in which was included a culture of *Elsinoë fawcetti* (strain 776A) from Sicilian lemon (table 2). Only potato-dextrose agar was used, and four tubes of each strain were prepared. The constant temperatures employed and the average diameter of the growth produced in 1 month are given in table 2, and representative cultures are shown in plate 11,

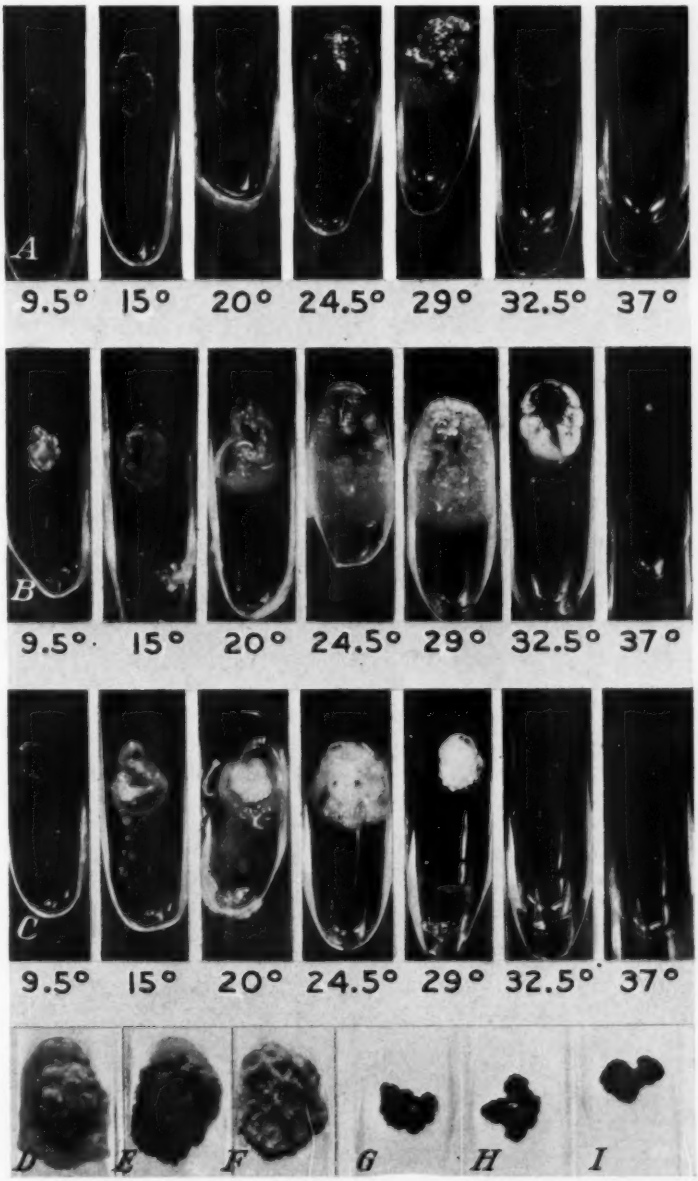
[15] This strain was obtained by Anderson and Kadow from the Department of Plant Pathology at Cornell University (*1*). It is a single ascospore isolation from Cumberland black raspberry made by Jones in 1922, and bears the number 340 of the Department of Plant Pathology, University of Wisconsin. A subculture was obtained by Jenkins and in 1927 a transfer from this was deposited in the culture collection of the Department of Plant Pathology at Cornell University.



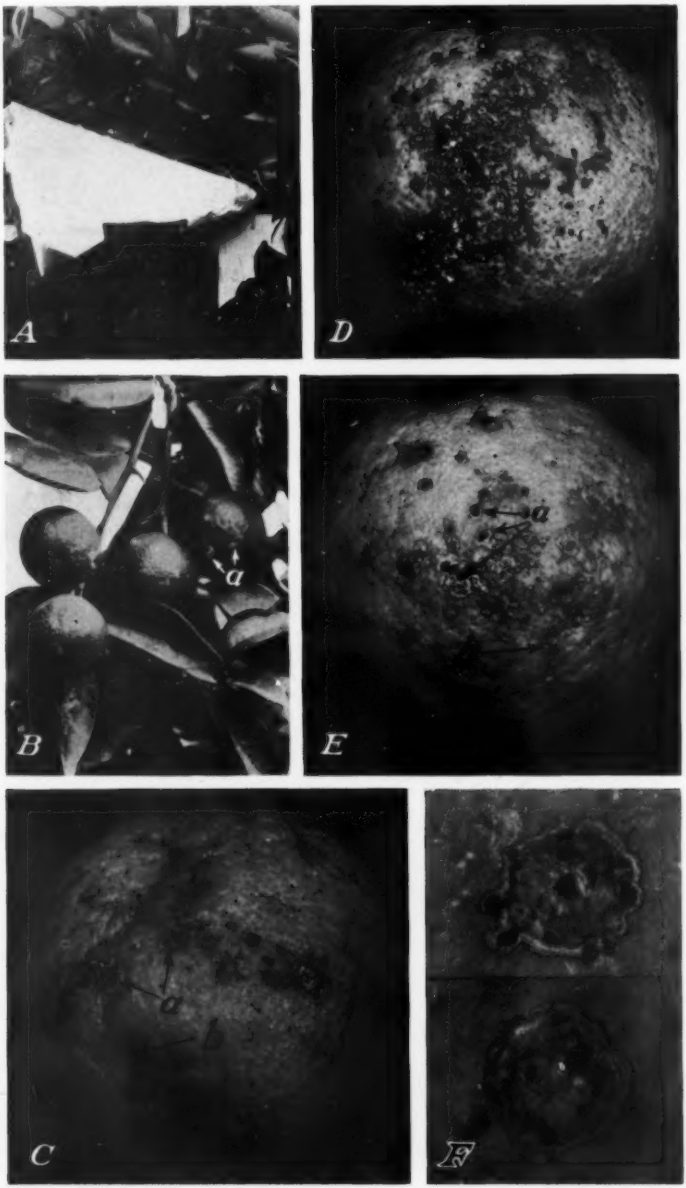
A–D, Comparison of *Elsinoë australis* (*A* and *B*) with *E. fawcetti* (*C* and *D*) on potato-dextrose agar; sources and arrangement of strains given in table 1; cultures in *A* and *C*, 12 days old; *B* and *D*, same cultures 26 days old; *E*, *F*, old cultures of *E. australis* (strain 375), and *G*, of *E. fawcetti*, showing growth from secondary colonies, *E*, on wort, *F* and *G*, on potato-dextrose agar. All × 1.



Old cultures of *Elsinoë australis* grown in Kolle flasks. × 1. *A*, Typical growth of strain 381B; spherical granules or secondary thalli shown at *a*. *B*, Saltant from 381B; *C*, lower side of same culture. *D*, *a*, Typical dark growth of strain 702A, together with a saltation, *b*; *E*, reverse of culture.



A and *B*, Potato-dextrose agar slant cultures of *Elsinoë australis* held for 1 month at indicated temperatures (° C.). *A*, Strain 702A, representing dark-colored growth of group 2; *B*, strain 381B, representing light-colored growth of group 1. *C*, *E. fawcetti*, strain 776A. *D*, *E*, and *F*, strains 345, 375, and 379 of *E. australis* grown at room temperature; *G*, *H*, and *I*, same strains grown at a cooler temperature. All × 1.



A, Triangular celluloid box employed in inoculating citrus fruits (× ¼). *B–F*, Infection of sweet orange fruits of the variety Abacaxi inoculated with *Elsinoë australis*; *B*, photographed 3 months after inoculation (× ¼); *C*, *D*, and *E*, same fruits 1 year after inoculation (× 1); *F*, enlarged primary lesions (× 5).

A, *B*, and *C*. In this experiment the optimal temperature range for *E. fawcetti* was 20° to 24.5° C., the optimal temperature probably being near 21°. These results are in general agreement with those previously reported for this fungus (*16*, *32*, *39*). Compared with each other, the two strains of *E. australis* were in practical agreement in their temperature relations, although, as usual, culture 381B covered a greater area of the slant. The maximum growth for each strain occurred at 24.5° to 29°, the optimum probably being near 26°, as in the first experiment. There was thus a difference of approximately 5° C. between the optimal temperatures for strains of *Elsinoë fawcetti* and those of *E. australis*. At 32.5° the two fungi were readily separable by the fact that the former species produced slight growth and the latter good growth.

In a third experiment all the cultures available were grown for 1 month at 32.5°. The results obtained for the 14 strains of *E. fawcetti* and for 22 of the 25 strains of *E. australis* were in harmony with those obtained in the experiment just described, that is, *E. fawcetti* produced practically no growth and *E. australis* good growth. Of the three other strains of *E. australis* at this temperature, one produced no growth and another slight growth, while the third gave a larger amount of growth than any of the other cultures at this temperature and was different in appearance.

The results of these experiments doubtless have important practical applications. For example, other conditions being favorable, it would seem that *Elsinoë australis* might occur in districts with temperatures too high for *E. fawcetti* at the time of fruit setting or at other times of the year. Moreover, if introduced into districts where *E. fawcetti* occurs, this fungus might become established there, since it also grows at the lower temperatures favorable to *E. fawcetti*. It is therefore evident that the conclusions of H. S. Fawcett (*16*) and of Peltier and Frederich (*32*), based on the temperature relations of *E. fawcetti*, may not be applicable to *E. australis*.

INOCULATIONS

Inoculation experiments on sweet orange, variety Abacaxi, with cultures of *Elsinoë australis* showed clearly that this fungus is the cause of sweet orange fruit scab. These experiments were made in a small experimental grove at Cantareira (near São Paulo). The disease was entirely absent when the first inoculations were made, October 10, 1932, and since the introduction of the fungus in this manner there have been discovered only a few lesions on fruits not artificially inoculated. In addition to the inoculations on sweet orange with *E. australis*, certain others were made in order to obtain the comparative data shown in table 3.

The method of inoculation, adapted from Winston (*39*, *p. 32*) in his inoculation experiments in Florida with *Elsinoë fawcetti*, is as follows: Macerated cultural inoculum spread on wet cotton squares is applied to young fruits less than 1 cm in diameter or to young leaves from 1 to 3 cm in length. This is covered with another thin layer of cotton. Each cluster of fruits or leaves thus inoculated is wrapped in wet absorbent paper and then encased in a triangular celluloid box (pl. 12, *A*). From each cluster a few fruits corresponding to those inoculated are employed as controls.

TABLE 3.—*Results of inoculating young fruits of sweet orange, tangerine, and laranja cravo and young leaves of sour orange and avocado with cultures of Elsinoë australis, E. fawcetti, and Sphaceloma perseae*

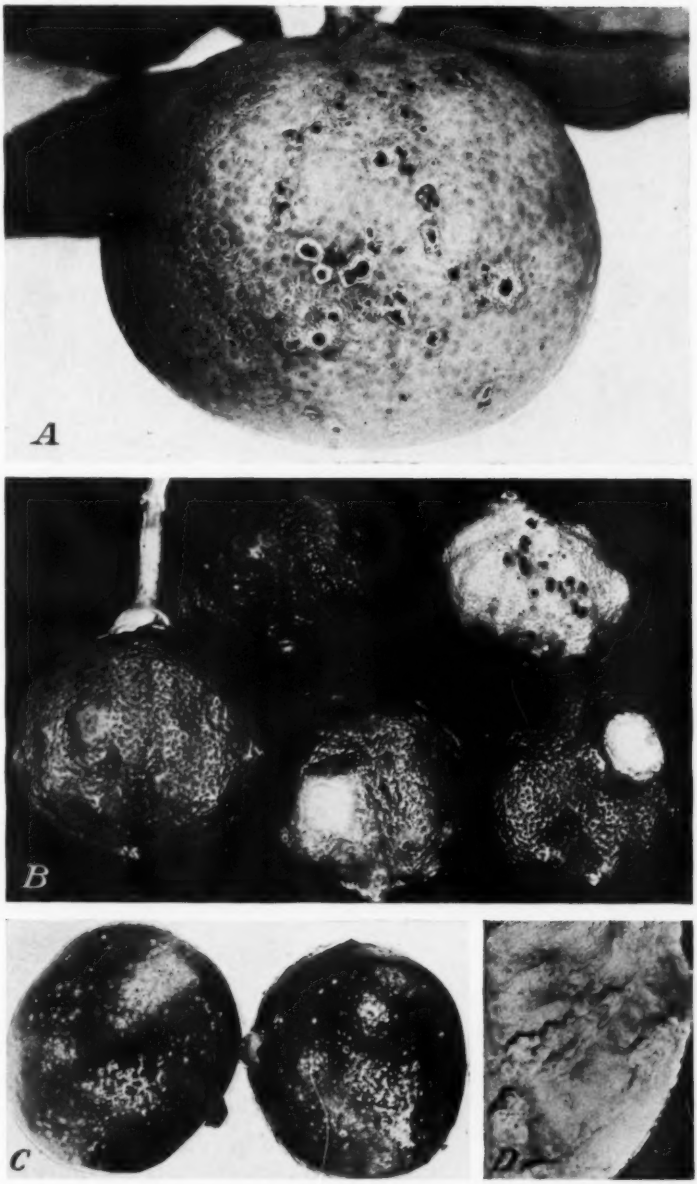
Test no.	Inoculum		Fruits or plants inoculated		Positive infections
	Source and strain no.[1]	Organism	Name	Number	
					Number
	Sweet orange (702A and 822A)	*E. australis*	Sweet orange (fruits)	10	8
	Different kinds of citrus, but not sweet orange or tangerine.	*E. fawcetti*	do	12	0
	Avocado (830A)	*S. perseae*	do	6	0
1	Sweet orange (702A and 822A)	*E. australis*	Tangerine (fruits)	8	8
	Sweet orange and tangerine (702A, 822A, and 933A).	*E. australis*	Sour orange (leaves) [2]	24	0
	Different kinds of citrus, but not sweet orange or tangerine.	*E. fawcetti*	do	16	1
	Avocado (830A)	*S. perseae*	do	2	0
	Sweet orange fruits (822A)	*E. australis*	Sweet orange (fruits)	5	1
	Sour orange leaf (128B)	*E. fawcetti*	do	[3]	
	Avocado (830A)	*S. perseae*	do	6	0
	Sweet orange fruits (822A)	*E. australis*	Laranja cravo (fruits)	6	[4] 6
2	Sour orange leaf (128B)	*E. fawcetti*	do	8	8
	Avocado (830A)	*S. perseae*	do	8	0
	Sweet orange fruit (822A)	*E. australis*	Avocado (leaves) [2]	20	0
	Sour orange leaf (128B)	*E. fawcetti*	do	20	0
	Avocado (830A)	*S. perseae*	do	40	26
3	Sweet orange leaf (381B)	*E. australis*	Sweet orange (fruits)	4	4

[1] See table 1.
[2] 3 to 5 leaves inoculated on each plant used.
[3] All fruits dropped.
[4] An isolation (strain 519B) was made from this set of inoculations.

Owing to conditions not connected with the experiment, heavy shedding of the fruits occurred in each of the three tests, greatly reducing the number of fruits on which infection readings could be made. It will be noted that *Elsinoë australis* gave infection on tangerine and laranja cravo as well as on sweet orange (pl. 12, *B–E;* pl. 13, *A* and *B*) and that all but 6 of the 33 inoculations with this fungus gave positive results (table 3). No infection resulted from inoculations on sweet orange with *E. fawcetti* or on sour orange with *E. australis*. Both fungi infected laranja cravo. Neither infected avocado, and *Sphaceloma perseae* gave infection only on avocado. In every case the lesions produced were typical for the disease represented. The controls all remained healthy. The single plant of sour orange that became diseased from inoculation with *E. fawcetti* showed good infection (pl. 13, *D*). On the other plants of sour orange absence of infection with this fungus was of course due to some unfavorable condition.

The lesions produced by *Elsinoë australis* on fruits of laranja cravo were larger and more prominent than those produced by *E. fawcetti*. They were also darker, owing chiefly to the presence of the black growth of the causal fungus on the lesions (pl. 13, *B*). The fruits of laranja cravo inoculated with *E. australis* were gathered 2 months after inoculation, whereas those inoculated with *E. fawcetti* were left on the tree for 2 months more. The latter fruits showed more severe infection at the end of that time than they had 2 months previously, indicating secondary infection (*C*). *E. australis*, as reisolated from the laranja cravo fruits inoculated with this fungus, constitutes the first of several reisolations of the organism. Some of these are from inoculations made with cultures from ascospores.[16]

[16] Unpublished data.



A and *B*, Infection of fruits of (*A*) tangerine and (*B*) laranja cravo, both inoculated with the same strain of *Elsinoë australis*, *A*, photographed 9 months, and *B*, approximately 2 months after inoculation. Both × 2. *C* and *D*, Infection of fruit of laranja cravo and leaf of sour orange, both inoculated with *E. fawcetti*; *C*, photographed approximately 4 months, and *D*, approximately 3 months after inoculation. *C*, × 1; *D*, × 2½.

Sweet orange fruits inoculated with *Elsinoë australis* became infected in about 10 days, and the lesions were limited in number. Such primary lesions on immature and on ripe fruits are shown in plate 12, *B*, *a*, and *F*. Primary lesions (*a*) from which secondary lesions (*b*) have evidently been formed are shown in *C* and *E*. Under grove conditions the primary infections may likewise be limited in number, as has been observed in the case of very young fruits. There are often not more than four or five primary lesions, but they are unusually large and prominent, and fructifications of the pathogen are present practically from the first. After a month or so the number of scabs on a given fruit may have greatly increased, evidently as a result of secondary infection from these primary lesions. A clear distinction between large primary and small secondary lesions is apparent when conditions are unfavorable for infection immediately following initial infection; otherwise the primary scabs are still being produced when the first secondary infections occur, and the resulting scabs are of all sizes. In nature the production of secondary lesions assists in explaining the existence of heavy infection despite the supposedly limited amount of inoculum at the beginning. This inoculum almost certainly comes from the following sources: (1) The comparatively small number of leaves showing infection; (2) out of-season green scabbed fruits; (3) ripe scabbed fruits left on the tree from the previous crop; and (4) twig infection.

SUMMARY

This paper deals with the sweet orange fruit scab, as yet identified only as it is found in South America.

The following sweet orange varieties are shown to be affected in São Paulo: Bahia Navel, Pera, Sabará, Selecta, São Sebastião, Santos, Lima, and Mangaratiba. The Abacaxi variety also has been proved to be susceptible. The disease has been observed in São Paulo on other kinds of citrus, for example, the tangerine of Brazil, tangerona, sweet lime known as lima da Persia, a sour lime known as limão seda, an unidentified citrus variety called laranja cravo, and a pointed-leaf papeda. In Argentina the sweet orange varieties, Ruby Blood, Sweet Mediterranean, Valencia, and Criolla, are known to be affected.

Sweet orange fruit scab has been identified from Argentina, Paraguay, Uruguay (apparently), and Brazil. In Brazil it is found in the States of São Paulo, Rio Grande do Sul, Minas Gereas, Rio de Janeiro, and in the Districto Federal. The earliest records of the disease are from Paraguay in 1882, São Paulo in 1900 (apparently), and Rio Grande de Sul in 1923.

The disease is of economic importance particularly because it blemishes the fruit. Beginning with the exportation of oranges to Europe (about 1929) Federal regulations were inaugurated in Brazil to prevent the shipment of low-grade fruit. The fruit scab then became a matter of concern to citrus growers of São Paulo. In some groves from 50 to 60 percent of the fruit was found blemished by the disease, some of it severely, and in numerous other groves as much as one-third of the crop was unfit for export.

The disease affects chiefly the fruits, rarely the leaves and twigs. It has the general aspect of sour orange scab, although there are appreciable differences in the character of the lesions. The symptoms of the disease are described.

In 1933 the causal fungus was described as *Sphaceloma fawcetti viscosa*. In 1936 the perfect stage was discovered and has been described as *Elsinoë australis* and the conidial stage designated *Sphaceloma australis*. The conidial stage is not characterized by the Cladosporiumlike development of *E. fawcetti* and *S. perseae*. Numerous persistent acervuli and sporodochia are produced on the lesions. They are warm buff to clove brown and fuscous-black.

Parallel cultural comparisons have shown that *Elsinoë australis* may be distinguished culturally from *E. fawcetti* and that the strains of *E. australis* may be divided into two main groups. Saltations may be produced, and these have been isolated and cultured separately.

In cultures grown at different constant temperatures, between 3.5° and 39.5° C., both *Elsinoë australis* and *E. fawcetti* grew from 9.5° to 35°. The best growth of *E. australis* occurred at 24.5° to 29°, the optimum probably being near 26°; while the best growth of *E. fawcetti* occurred at 20° to 24.5°, the optimum probably being near 21°.

In inoculation experiments *Elsinoë australis* from sweet orange gave positive results on sweet orange, tangerine, and laranja cravo. The organism was reisolated. *E. fawcetti* infected laranja cravo and sour orange, but not the sweet orange. *Sphaceloma perseae* gave good infection on avocado. Cross inoculations with this fungus on citrus gave negative results, as did cross inoculations on avocado with *E. fawcetti* and *E. australis*.

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ORGANIZATION OF THE UNWALLED ASCUS IN TWO SPECIES OF CERATOSTOMELLA [1]

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INTRODUCTION

The ascus, characteristic of a major group of the fungi known as the Ascomycetes, is a familiar example of a type of reproductive body or special plant structure in which a number of independent cellular units are produced within the body of a walled parent cell. The unique process of free cell formation, by which ascospores are delimited within the parent cytoplasm, is commonly believed to distinguish the ascus from all similar plant structures. The investigations of Harper (*14*, *15*)[3] and his students, Sands (*24*), Schultz (*25*), and others on the cytology of the ascus have strongly influenced the views of present-day botanists in regard both to the nature of the ascus and to questions of homology of reproductive bodies in the lower plants. The work of these investigators, while in many respects comprehensive, does not represent adequately those species of fungi in which the asci are said to be "deliquescent."

Early disintegration or deliquescence of the ascus is a characteristic encountered in representatives of many genera of Ascomycetes, particularly those of the order Sphaeriales. The recent work of Mittmann (*21*) and of Andrus and Harter (*1*) on *Ceratostomella fimbriata* (Ell. and Hals.) Elliott has indicated that deliquescence of asci is not necessarily a result or a process of wall dissolution, since the ascus of *C. fimbriata* possesses no wall from the time of its origin. Deliquescence of the ascus seems to represent a disorganization of the peripheral region of a naked or plasmodial mass that has already reached a state of senescence. The same investigators observed the significant fact that the central spore-producing region of the ascus of *C. fimbriata* is vesiculate and sometimes distinctly walled, a condition not comparable to anything described in the more familiar type of ascus.

The early history of the unwalled cells that later become transformed into asci in *Ceratostomella fimbriata* further distinguishes this organism from the more familiar type of ascomycete. Although there is remarkably close agreement in the accounts given by Mittmann (*21*), and by Andrus and Harter (*1*) concerning the fragmentation of the ascogonium in *C. fimbriata* and the continuous divisions of the ascogenous cells, these writers have neglected to describe in sufficient

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[2] The writers acknowledge with gratitude the valuable assistance and criticism rendered at various stages in the investigation by A. E. Longley, Division of Cereal Crops and Diseases, W. W. Diehl, Division of Mycology and Disease Survey, and C. Drechsler, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and by R. F. Griggs, professor of botany, George Washington University.

[3] Reference is made by number (italic) to Literature Cited, p. 44.

detail the actual divisions of the isolated cells and the nuclear behavior in the ascus. The object of the present paper is to give a more complete picture of cell and nuclear behavior involved in the formation of asci of the *C. fimbriata* type and to compare these phenomena with those described in certain other fungi. This will be accomplished by describing additional observations on *C. fimbriata* and will be supplemented by a study of *C. moniliformis* Hedge.

MATERIAL AND METHODS

At the present time, in addition to species with deliquescent or unwalled asci, the genus *Ceratostomella* includes species in which the asci possess definite and persistent walls. Also included in this genus are species that possess an imperfect stage of the endoconidial type and various other very distinct types of conidial fructifications. So far as known all species with an endoconidial stage also possess asci of the deliquescent type. Recently Melin and Nannfeldt (*20*) have tentatively proposed to transfer to the genus *Ophiostoma* Sydow those species in which asci are deliquescent and to retain the genus *Ceratostomella* for species with persistent asci. Still more recently it has been proposed by Davidson (*4*) to segregate the endoconidial species into the genus *Endoconidiophora* Münch. Although there seems to be real justification for dividing the species of *Ceratostomella* among three different genera, the organisms used in the present investigation will be called by their more familiar names.

Ceratostomella fimbriata was isolated from sweetpotatoes, where it causes the disease known as black rot. Its taxonomic history has been reviewed in a previous publication (*1*). It has been the subject of morphological investigations by Elliott (*6*), Mittmann (*21*), and Andrus and Harter (*1*). *C. moniliformis* was isolated from lumber, but it does not seem to be an active agent in wood decay or blue stain. Both fixed and living material of the latter organism were supplied the writers by R. W. Davidson, of the Bureau of Plant Industry, United States Department of Agriculture. Additional fixations were made from the live cultures.

The two species included in this study are readily cultured on potato-dextrose agar, Thaxter agar, and malt agar media. Fixations were made from Thaxter agar cultures on the third day of growth in the case of *Ceratostomella moniliformis*, and on the sixth day with the less rapidly growing *C. fimbriata*. Material was embedded in commercial Parawax and sectioned 4μ to 6μ in thickness with a rotary microtome. Land's gum arabic-potassium bichromate method was used in attaching ribbons to the slide.

The following combinations of killing solution and stain were used with each of the species: Bouin's solution followed by the triple stain (safranin, gentian violet, and orange G); Bouin's solution followed by Heidenhain's haematoxylin; Flemming's solution followed by triple stain; and Flemming's solution followed by Heidenhain's haematoxylin. In addition, a small amount of material of *Ceratostomella moniliformis* was fixed in a formol-acetic solution [4] which was followed by the triple stain. The Flemming's solution was prepared after the stronger formula and then diluted with an equal volume of water. Some brilliant preparations were obtained from material fixed in this

[4] Prepared by adding 1.5 cc of glacial acetic acid and 8.5 cc of 40-percent formaldehyde to 90 cc of 50-percent alcohol.

solution and stained with the triple combination, yet they were in no way superior to the best results obtained when Bouin's fixative was used. Bouin's solution was used by Mittmann (*21*) in her work with *C. fimbriata* and by Andrus and Harter (*1*) in their investigation of the same species. Recently Raymond (*23*), in a comprehensive study of ascomycete cytology, recommended Bouin's in preference to solutions containing osmic acid. Although it had not previously been recommended for botanical work, recent experience demonstrates that Bouin's fixative can be used very successfully in the investigation of fungus morphology. Stages in nuclear division in *C. moniliformis* were observed more frequently in material fixed with the formol-acetic solution, yet for general purposes this solution seemed to be inferior to either Bouin's or Flemming's.

The various combinations of fixatives and stains were used to secure useful preparations and are not expected to indicate the relative merits of the several methods. The most generally useful preparations were those from material fixed in Bouin's solution and stained with haematoxylin. In some cases the haematoxylin alone proved satisfactory, yet various counter stains were tried (orange G, safranine, and erythrosin, or combinations of these three), and in a few instances good effects were obtained.

Recent use of Heidenhain's haematoxylin has resulted in bringing out numerous details of cell and nuclear behavior in *Ceratostomella fimbriata* that were lacking in previous publications on the morphology of the species. The earlier inability to observe certain stages in ascus formation apparently has not been due to the use of an inadequate fixative but has resulted from a failure to secure an adequate stain. It will be evident that a great many details of the development in each of the two species discussed herein are still lacking, yet to a considerable extent those available for one species may compensate for those lacking at a corresponding stage in the other. At the same time it will not be possible to overlook certain characteristic differences in cytological behavior.

ORIGIN OF THE BINUCLEATE CONDITION IN CERATOSTOMELLA

Early stages in the development of the fruiting body and the accompanying appearance of the binucleate condition in the ascogonium have been described previously in *Ceratostomella fimbriata* (*1*, *21*). Corresponding stages in the development of *C. moniliformis* are similar to *C. fimbriata*. The earliest appearance of the fruiting body can be recognized in living cultures as a short hyphal outgrowth with a thin-walled recurved tip (fig. 1, *A–H*). After fixation and application of stain, the cells involved in the primary structure are seen to possess each a single nucleus (*I*). The terminal portion of the recurved hypha develops into a coil of several cells. The point *a* of figure 1 marks approximately the region in which the binucleate condition first appears and which later gives origin to the multitude of asci. Septa do not usually appear in the terminal portion of the coil until the latter has been enclosed by enveloping hyphae (*J*, *K*).

The manner in which the binucleate condition arises has not been definitely determined. There is evidence of an antheridium in both species, but no satisfactory evidence that the latter organ fuses with

the ascogonium. Both species are homothallic, since the fruiting bodies or perithecia are produced abundantly in cultures from single spores. Although there is the possibility of a true fertilization by fusion of antheridium and ascogonium, by "conidiation" (*5*), or by some other form of anastomosis involving nuclear migration, yet it seems probable that development of the fruiting body is apogamous.

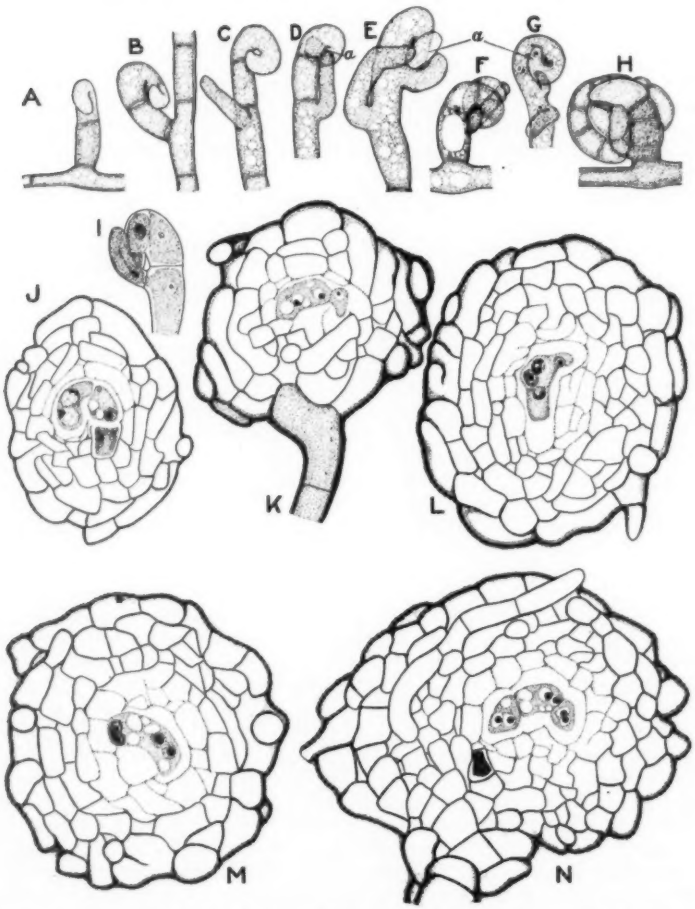

FIGURE 1.—Development of perithecia in *Ceratostomella moniliformis: A–H*, Perithecial primordia from live material; *a*, approximate location of region in which binucleate condition first appears. *I*, Primordium from stained section. *J–L*, Young perithecia, showing origin of paired nuclei in the ascogonium. *M*, *N*, Young perithecia, showing the first divisions of the binucleate portion of the ascogonium. × 1500.

The best available evidence indicates that the binucleate condition first appears as shown in figure 1, *L*. Apparently two successive nuclear divisions in the terminal portion of the ascogonium are followed by two cleavage planes that cut off a uninucleate terminal and basal cell and leave the subterminal cell with two nuclei. This is es-

sentially a crosier device—an indirect type of cell cleavage originally described by Dangeard (*3*)—but there is no indication that the first and third cells fuse as in a true crosier. The crosier type of cell division, however, appears at a later stage, i. e., just previous to ascus formation.

Immediately after the first layers of enveloping hyphae are in place, the original wall of the ascogonium begins to disintegrate, although remnants of the wall can still be recognized at slightly more advanced stages. Following the complete dissolution of the wall, the cells of the ascogonium have the appearance of essentially naked protoplasts isolated in the small cavity of the perithecium. The unwalled condition persists throughout the period of cell proliferation and up to the time of ascospore maturation.

CELL DIVISIONS PREVIOUS TO ASCUS FORMATION

The first proliferation of the initial binucleate cell or fertile cell of the ascogonium in *Ceratostomella moniliformis* appears to follow a simple linear growth with divisions into binucleate daughter cells (fig. 1, *M*, *N*). The cells lose their linear relations but continue to divide independently, with the result that just preceding beak formation the perithecial cavity contains a multitude of binucleate cells that for the most part are isolated from one another and free from the wall of the perithecium. The early period of cell multiplication is distinguished to a slight degree from the later stage by the larger size of individual cells and nuclei and apparently by the absence of the crosier type of cell division. The corresponding stage of *C. fimbriata* has been described by Mittmann (*21*) and by Andrus and Harter (*1*). There is evidence in both species that uninucleate cells of the ascogonium undergo a limited and independent proliferation in the perithecial cavity. The presence of small scattered uninucleate cells with weak affinity for stain can be demonstrated in all early stages of perithecial development.

Approximately at the beginning of beak formation the perithecial cavity is seen to contain the first young asci and at that time also a change is noted in the appearance of the dividing cells. Individual cells frequently cling together in short chains or groups, and the nuclei are smaller though still quite distinct; but most characteristic of the later stage in development is the appearance of the crosier type of cell division. The various stages in this and other types of cell division in the two species are illustrated in figure 2, *B* to *V*, and figure 3, *A* to E_1.

Earlier observers (*1*, *6*, *21*) believed that the formation of crosiers or ascus hooks did not occur in *Ceratostomella fimbriata*. It is evident from the illustrations here presented that structures which are at least the equivalent of crosiers do occur in *C. fimbriata* and in *C. moniliformis* as well. Some authors have attached considerable importance to the occurrence of the crosier type of cell cleavage at a point immediately preceding ascus formation, but the crosier is only one of several types of cell division that may be observed preceding ascus formation in *Ceratostomella*. These proliferative stages are described in full in the following paragraphs.

The cells concerned in crosier formation in *Ceratostomella* are considerably modified to conform to their detached and unwalled condition. The resemblance to hook formation in ascogenous hyphae of

discomycetous species is in some instances very remote; this being especially true where no protoplasmic connections between the individual parts of the crosier remain. Also, in many instances, owing to the shape of the parent cell, there is no clear differentiation of terminal and basal regions, since the two portions are abstricted together. In such cases the process approaches that of a simple division of binucleate cells (fig. 2, *O*), and in fact it is evident that both the crosier type of cell division and simple cell cleavage (figs. 2, *A*; 3, *F*) occur side by side even in later stages of perithecial development.

There are three reasonably distinct types of cell division in perithecia of *Ceratostomella*. In one type, following division of the paired nuclei, the parent cell is divided into three portions by two cleavage planes (figs. 2, *E*, *L*, *P*; 3, *Z*). The central portion retains two nuclei, and each of the two terminal cells possesses a single nucleus. The two uninucleate portions usually approach each other (fig. 3, *K*) and fuse (fig. 3, *L*). This differs in no important respect from the crosier device of discomycetous species. The two binucleate cells resulting from such a division may cling together, as in figure 3, *E*, or they may become separated. In either case each cell may continue to divide independently, or one or both may develop into an ascus (fig. 3, *G*).

In the second type of cell division the four-nucleate body (fig. 3, *C*), following division of the paired nuclei, is divided into three portions by three planes of cleavage. Here also there would seem to result a central portion with two nuclei and two uninucleate basal cells (*U*, *W*). But at the third point (*V*, *a*) cleavage does not seem to be completed, so that there actually results a pair of binucleate cells. Then follows a reunion of the two basal portions which apparently were never entirely separated. In the second type of division, also, the two binucleate fragments may continue to divide independently, or one or both may develop into an ascus.

In the third and simplest type of cell division, a single cleavage (figs. 2, *A*, *G*; 3, *D*, *F*) or two confluent planes of cleavage (fig. 3, A_1, C_1) divide the four-nucleate body into two binucleate portions, each of which may continue to divide independently.

The two uninucleate cells formed during crosier formation may remain separated, sometimes individually as an appendage of a fertile ascus (fig. 3, F_1). They may develop for a time independently, and eventually join the disorganized material in the center of the perithecial cavity. Frequently structures in *Ceratostomella fimbriata* that appear to be abortive asci (see fig. 7, *M*, *R*) may have originated from uninucleate portions of the crosier, while in *C. moniliformis* numerous

EXPLANATORY LEGEND FOR FIGURE 2

A, Direct cleavage of a binucleate ascogenous cell; *B*, Nuclear division in the crosier; the position of spindles indicates that a direct cell cleavage will follow. *C*, *D*, Nuclear divisions in the crosier, showing characteristic cell shape and divergent position of spindles; *E*, Approximation of uninucleate portions of a crosier; *F*, An appearance of budding that is probably equivalent to crosier formation; *G*, A unilateral form of direct cleavage; *H*, A four-nucleate stage in crosier formation; *I*, Two pairs of divergent spindles in an ascogenous cell of the crosier type; *J*, Nuclear division in the crosier; *K*, An early stage following nuclear division, with the original nucleoli still visible; *L*, *M*, Approximation of uninucleate portions of a crosier; *N*, *O*, The four-nucleate stage in crosier formation; *P*, An early indication of cleavage planes in a crosier; *Q*, *S*, Crosier formation and cell linkage; *T*, Change in cell shape previous to cell division; *U*, Fusion of uninucleate portions of a crosier; *V*, Linked components of a crosier in one of which nuclear division has again occurred; *W*, *X*, Nuclear fusion in the ascus; *Y*, *Z*, A_1–D_1, Linkage of young asci; E_1, An abortive ascus or sterile body; F_1, Linkage of asci; G_1, H_1, Nuclear fusion in the ascus; I_1, J_1, Stages preparatory to the first nuclear division in the ascus; K_1–P_1, Early stages in the first nuclear division in the ascus, showing the emergence of the nucleolus from the nuclear vesicle and emphasizing the vacuolation of the peripheral region of the ascus that accompanies increase in cell size; Q_1, A first-division figure showing four discrete chromatin bodies and the persistent nucleolus; R_1, A later stage indicating the movement of two chromosomes or a single bilobed chromatin body to each pole; All × 3000.

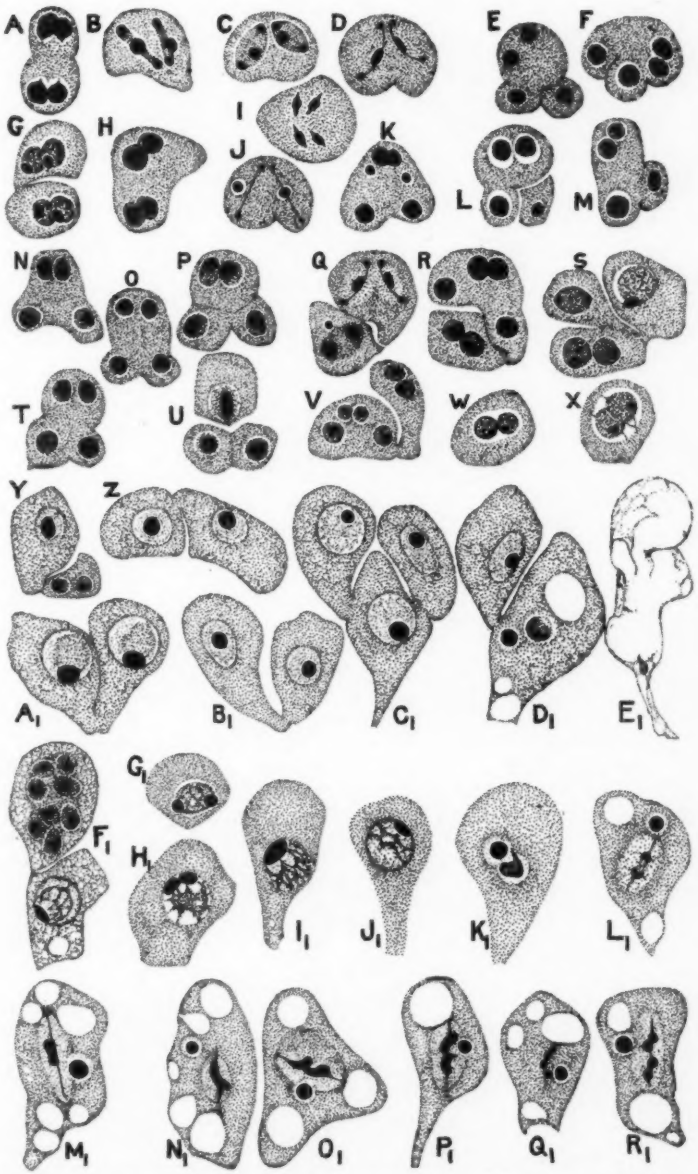

FIGURE 2.—Cell and nuclear division in *Ceratostomella moniliformis*. (For explanatory legend see opposite page.)

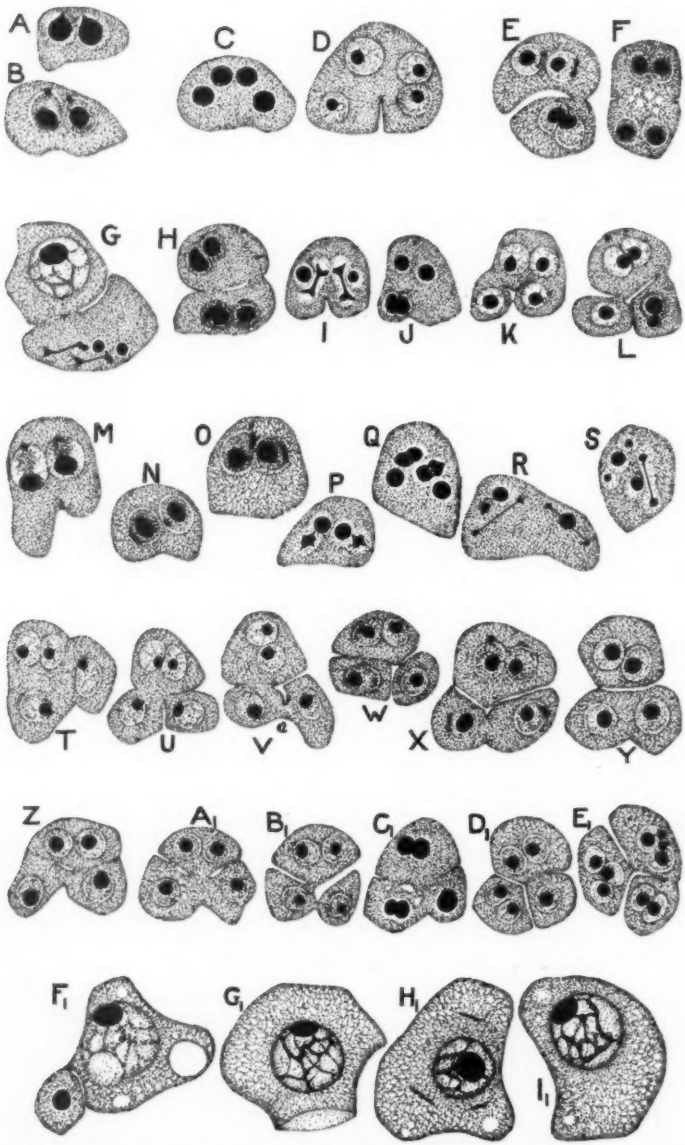

FIGURE 3.—Cell and nuclear division in *Ceratostomella fimbriata*. (For explanatory legend see opposite page.)

fragile, inflated, and apparently uninucleate cells that precede the fertile asci in the center of the cavity (figs. 2, E_1; 4, *L*; 6, *E*) may have had a similar origin. The weak haploid cells sometimes display a series of nuclear changes that seem to correspond to those that take place in a fertile ascus.

It may be necessary to distinguish two types of haploid cells in the perithecial cavity, namely, those resulting from chance irregularities in the division of binucleate ascogenous cells and those that have developed independently from uninucleate portions of the original ascogonium. The former could be regarded as haploid asci, while the latter are perhaps homologous with paraphyses of discomycetous fungi.

There is ample evidence that the uninucleate components of the crosier sometimes fuse to form a second binucleate cell (figs. 2, *U*; 3, *X*) which undergoes further division. It appears that the originally binucleate portion of the crosier usually, but not invariably, develops directly into an ascus, whereas the portion resulting from fusion of the uninucleate proximal cells continues to divide. Evidence for this is based largely on the shape and position of cells during and after crosier formation.

Change in cell shape plays an important role during the process of cell division. The changes in conformation are made conspicuous by the circumstance that a wall, commonly present on ascogenous hyphae of discomycetous species, is lacking on ascogenous cells of *Ceratostomella*. The cells undergoing rapid multiplication within the perithecium correspond conceivably to the protoplasmic contents of septate hyphae after the hyphal walls have been dissolved away, and the slender filaments that frequently provide a connection between individual cells (figs. 2, F_1; 7, *A*) perhaps correspond to the plasmodesma that extend through the perforations in hyphal cross walls.

Change in cell shape will account for the fact that a cell of the kind shown in figure 2, *D*, will later appear as in figure 2, *N* and *T*, without any appreciable increase in cell size. By change in cell shape two distal portions of a crosier are brought together and fused. Also, the appearance of budding shown in figure 2, *F* and *M*, may be produced by change in shape of the cell and not necessarily by a true growth process. It is not intended to imply that the cells undergo amoeboid movements. The movements that produce the changes in shape probably are made slowly, and the fact that such movements actually take place is only inferred from the appearance of the cells in the fixed and stained material.

EXPLANATORY LEGEND FOR FIGURE 3

A, An ascogenous cell immediately following cell division, showing the reappearance of nucleoli and "centrosomes"; *B*, Reorganized nuclei in young crosier, showing the usual position of a satellite or centrosome at the margin of each nuclear vesicle; *C*, A typical crosier following conjugate nuclear division; *D*, A stage in unilateral cell cleavage; *E*, Cell linkage following an apparently unilateral cleavage; *F*, A stage in simple and direct cell division; *G*, Cell linkage. The parallel position of spindles may indicate that a direct cell cleavage will follow; *H*, A stage in cell division that may have been direct or indirect; *I*, Spindle formation in the crosier; *J*, A stage in one type of direct cell division; *K*, A crosier at the four-nucleate stage; *L*, Fusion of uninucleate portions of a crosier; *M–O*, Crosiers with paired nuclei prepared for division; *P–S*, Spindle formation in the crosier; *T–Y*, Stages in crosier formation; *V*, *a*, Point of incomplete cleavage resulting in pair of binucleate cells; *Z*, A_1–C_1, Stages in cell division brought about by two confluent planes of cleavage; D_1, E_1, Cell cleavage accompanied by an irregular distribution of nuclei; F_1, A young ascus with an appended uninucleate cell fragment; G_1–I_1, Nuclear structure previous to the first division in the ascus. All × 3000.

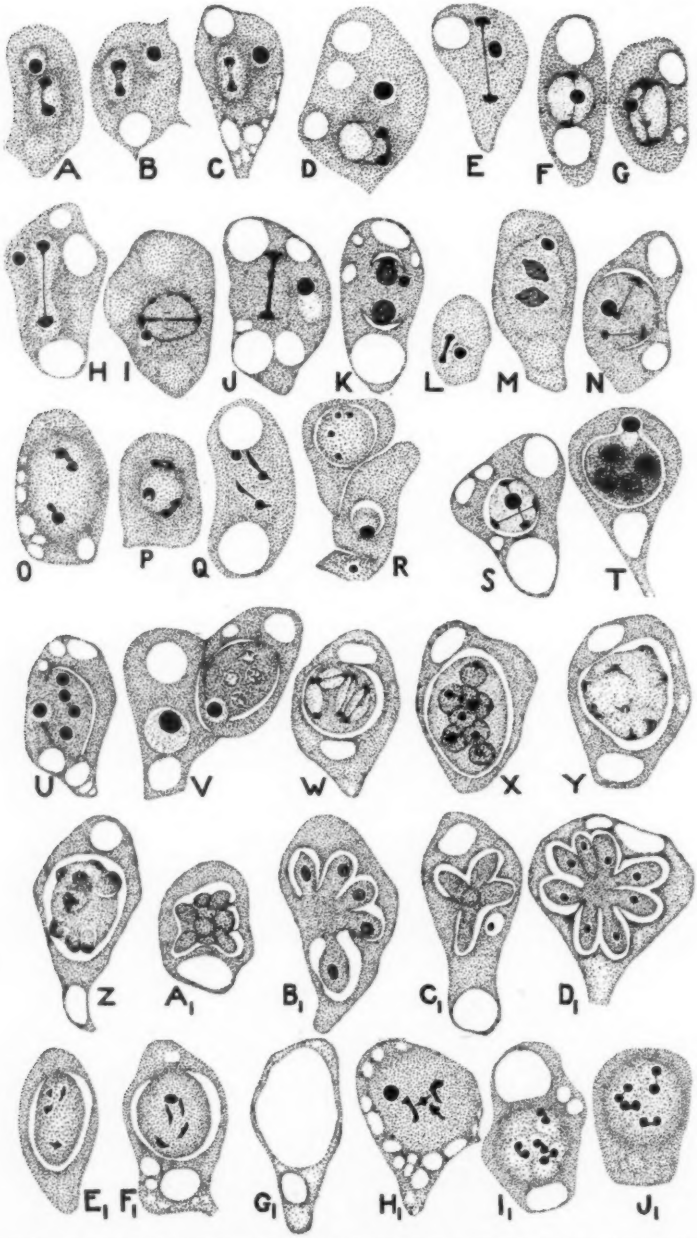

FIGURE 4.—Nuclear division and ascus formation in *Ceratostomella moniliformis*. (For explanatory legend see opposite page.)

Characteristic bodies that appear to behave like centrosomes are present in prefusion nuclei of both *Ceratostomella moniliformis* and *C. fimbriata*. They are more conveniently observed in the latter species. The single chromatic granule situated conspicuously on the periphery of each nucleus (fig. 3, *B*, *M*) appears to become attenuated and bilobed just preceding nuclear division (*E*, *N*, *O*). The spindle-shaped structure at first traverses the nuclear vesicle, but later each end of the spindle extends beyond the limits of the vesicle. Nuclear division seems to take place along the slender axis. The nucleolus during this time is situated usually midway of the spindle (*I*, *S*) and remains visible until division is complete. Following nuclear division the old nucleolus dwindles in size and commonly disappears before cell cleavage. At the same time a new nucleolus, together with a satellite or centrosome, appears in the reorganized nucleus (*A*).

NUCLEAR STRUCTURE AND NUCLEAR FUSION

CERATOSTOMELLA MONILIFORMIS

In *Ceratostomella moniliformis* the apparent absence of a nuclear membrane is a notable characteristic of prefusion nuclei. The nuclei commonly have the appearance indicated in figure 2, *G*, *H*, with the limits of the nuclear vesicle quite indistinct. At the time of nuclear fusion the two vesicles become confluent, so that at a certain stage two nuclei appear to be floating in a single hyalosphere (*W*). The outline of the hyalosphere becomes more distinct at this time, although a well-defined nuclear wall rarely is seen. A mingling of the two separate spheres of nucleoplasm (*X*, G_1) marks the second stage in nuclear fusion, and a fusion of the two nucleoli (H_1, I_1) indicates completion of this stage in ascus formation.

While the content of prefusion nuclei appears to be homogeneous, with affinity principally for plasma stains, the fusion nucleus possesses an elaborate network to which chromatin stains adhere (fig. 2, I_1). The comparatively large and frequently lens-shaped nucleolus of the young ascus (A_1) commonly assumes a position between the central body of nucleoplasm and the margin of the nuclear vesicle. In this condition the whole structure, including the cell body, increases in size. Just previous to or during the first nuclear division in the ascus characteristic large vacuoles appear peripherally in the cytoplasm (M_1–R_1).

EXPLANATORY LEGEND FOR FIGURE 4

A–H, First nuclear-division stages that in most cases show evidence of a persistent nuclear vesicle; *I*, stage in spindle formation, with chromatic granules or inclusions marking the periphery of the original nuclear vesicle; *J*, an advanced spindle with evidence of a persistent membrane at each pole; *K*, reorganization of daughter nuclei following the first division, and showing an early appearance of cleavage at points originally occupied by the definitive nuclear membrane; L_1, nuclear division in a probably haploid ascus; *M*, beginning of the second nuclear division in the ascus; *N*, spindles during the second nuclear division traverse the contents of an inner vesicle which at this stage becomes conspicuously separated from the outer body of the ascus; *O–Q*, second-division stages after formol-acetic fixation; *R*, linkage of asci, with an appended uninucleate fragment; *S*, contraction of the interior region of the ascus at the second nuclear division; *T*, *U*, the four-nucleate stage in ascus formation; *V*, linkage of asci in a manner to indicate that cell cleavage was incomplete; *W*, the third nuclear division in the ascus; *X*, the eight-nucleate stage in ascus formation; *Y*, *Z*, emergence of the eight nuclei on the surface of the spore-producing mass suspended in the ascus vesicle; A_1, outgrowth of spore rudiments from the surface of the vesicle contents; B_1–D_1, continued growth of spore rudiments into the outer cytoplasmic body of the ascus, with indication that the spores extend from a common base; E_1, F_1, the four-nucleate stage, showing the vesiculate structure of the ascus interior; G_1, an abortive ascus, or one from which the spore-producing contents have been displaced; H_1–J_1, asci during the third nuclear division, showing a frequent appearance of chromatic contents of the nuclei. All × 3000.

CERATOSTOMELLA FIMBRIATA

Ceratostomella fimbriata is similar to *C. moniliformis* in the structure of prefusion nuclei (fig. 3) and in the various stages of nuclear fusion. The structure of the fusion nucleus, however, deserves careful consideration because of the important role that the nuclear vesicle is believed to play during later stages of ascus formation.

The fusion nucleus possesses a network of nodose filaments that gather into thick strands just preceding nuclear division (figs. 3, G_1, I_1; 5, *B*) and form an increasingly compact plate of chromatic substance across the nuclear vesicle (fig. 5, *C*, *D*). The nucleolus becomes spherical instead of lens-shaped and withdraws from the nuclear vesicle. Unlike the nucleolus in *Ceratostomella moniliformis*, which persists throughout ascus formation, that in *C. fimbriata* disappears during the first nuclear division (fig. 5, *J*, *K*). Local concretions or thickenings sometimes observed at the periphery of the nucleus (fig. 5, *A*) may presage the later transformation of the nuclear membrane into an endogenous ascus wall, yet this is improbable since in many cases the original nuclear vesicle is distinguishable only by a faint outline during the first and second nuclear divisions.

ORGANIZATION OF THE ASCUS

FIRST NUCLEAR DIVISION

CERATOSTOMELLA MONILIFORMIS

In *Ceratostomella moniliformis* the beginning of the first division is indicated by the aggregation of chromatin threads in a crude plate across the face of the nuclear vesicle (fig. 2, J_1). The threads condense into an irregularly lobed or sometimes spherical mass of chromatin (D_1, K_1, M_1, N_1) that breaks into two portions situated midway on a simple rod-shaped axis (L_1, O_1, P_1). Later there are four reasonably distinct bodies distributed along the axis of the spindle (Q_1), two of which move to each pole. Often a single bilobed chromatin body appears to be drawn to each pole (R_1). The amount of chromatin substance passing to the opposite poles frequently appears unequal (fig. 4, *A*, *B*), but the resultant daughter nuclei as well as the spindles of the second division appear equal. The advanced spindle is a dumbbell-shaped structure (fig. 4, *E*, *H–J*) with a simple axis that rarely seems to consist of two filaments (fig. 4, *I*).

The number as well as the rather characteristic shape of the chromatin bodies distributed on the spindle has been observed repeatedly, and there seems little reason to doubt that these bodies correspond to the chromosomes described in other species of fungi. At the same

EXPLANATORY LEGEND FOR FIGURE 5

A, An ascus with local thickenings at the periphery of the definitive nucleus; *B*, Withdrawal of the nucleolus from the nuclear vesicle at the first stage in nuclear division; *C*, Gathering of the chromatic contents of the nucleus preparatory to division; *D*, Separation of chromatin bodies at metaphase; *E–I*, Metaphase figures showing chromosomes attached at various angles to the axis of the spindle; *J*, *K*, Four-lobed spindles at metaphase. The nucleolus is disintegrating at the margin of each nuclear vesicle; *L*, Anaphase, showing fusion of two chromosomes into a single chromatin mass at each pole; *M*, A typical figure at late anaphase, showing chromatin masses at each pole connected by a single distinct filament; *N*, A late metaphase, showing two pairs of chromosomes, each pair consisting of a simple and a compound chromatin body; *O*, A first-division figure at anaphase, with the old nucleolus still visible at the margin of the nuclear vesicle; *P*, Reorganized daughter nuclei following the first division in the ascus, showing their structure and arrangement in the vesicle; *Q–T*, The second nuclear division in the ascus; *U*, *V*, Eight-nucleate asci, showing the spore-producing region enclosed by a definite wall. All × 3000.

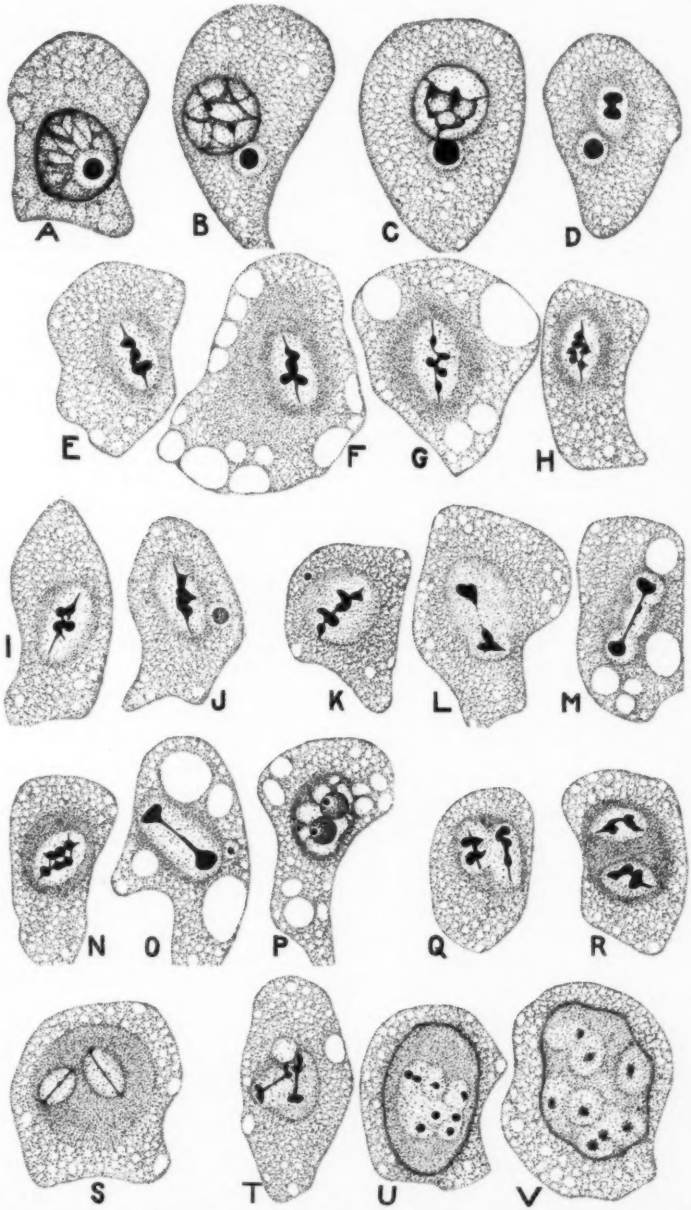

FIGURE 5.—Nuclear division and ascus formation in *Ceratostomella fimbriata*. (For explanatory legend see opposite page.)

time it is conceivable that discrete chromatin bodies in fungi may not be equivalent to chromosomes of higher plants. The latter viewpoint was expressed by some of the earlier workers in the field of fungus cytology (*10*), but the more recent workers have come to regard such a reservation as unnecessary.

The outline of the original nuclear vesicle usually remains visible during the first division in the ascus, although there seems to be no definite nuclear wall. Chromatic granules that appear in material killed with osmic acid sometimes indicate the margin of the vesicle (fig. 4, *I*).

The behavior of the nucleolus during the nuclear divisions in the ascus is a conspicuous and perhaps an important characteristic of the species. Just preceding the first spindle formation, the nucleolus withdraws to one side of the nuclear vesicle (fig. 2, D_1 K_1) and remains there during the nuclear divisions and up to the time of ascospore formation (figs. 2, L_1–R_1; 4, *A*–*N*, *S*–*V*; 6, *A*, *F*, *H*). It is surrounded by a faint or sometimes distinct halo, which, however, often appears to be continuous with the outline of the nuclear vesicle (figs. 2, O_1; 4, *M*, *T*). The persistence of the nucleolus in its relation to the vesicle (fig. 4, *T*–*V*) comprises part of the evidence upon which it is concluded that the ascospores are formed within the nuclear vesicle. In *Ceratostomella moniliformis* the vesicle appears to be without a wall, whereas in *C. fimbriata* a definite endogenous wall is frequently observed just previous to ascospore formation.

The reorganized daughter nuclei following the first division in the ascus are similar in size and general appearance to prefusion nuclei. An eccentric highly chromatic body, as indicated in figure 4, *K*, does not seem to be the equivalent of the nucleolus in the prefusion stage. Rarely at this time a break occurs in the cytoplasm of the ascus at a position corresponding to the margin of the original vesicle of the fusion nucleus. More often this cleavage of the cytoplasm does not occur until the second nuclear division is nearly completed.

Ceratostomella Fimbriata

In *Ceratostomella fimbriata* the first-division figure is enclosed by an elliptical hyalosphere within the limits of the original nuclear vesicle, which in many instances is indicated only by the regular contour of a central mass of denser protoplasm (fig. 5, *N*). There is little evidence that a centrosome is present during nuclear division in the ascus. While two denser points of granulation can be observed occasionally during early stages of division, the two poles of the advanced spindle (*E*–*I*) seem to merge into the cytoplasm at the margin of the hyalosphere.

During the first nuclear division in the ascus the spindle first appears as a continuous two-lobed mass of chromatin that later separates into four distinct bodies attached at various angles to a single filament or axis (fig. 5, *F*, *I*, *K*). In some figures five chromatic granules appear to be distributed along the axis (*E*, *G*); in other instances six may be seen (*H*, *N*). It will be observed, however (*H*, *N*), that the four bodies nearest the midpoint on the spindle appear as two dumbbell-shaped structures, each pair probably representing a single "chromosome." This arrangement of chromosomes, if such they may be called, seems to be characteristic. Two chromosomes, one oval or comma-shaped

and the other irregularly lobed, move toward each pole (*L*). They appear as a single mass of chromatin after they reach their respective poles (*M*, *O*).

Since the writers have found no evidence of a double nuclear fusion in *Ceratostomella fimbriata* or in *C. moniliformis*, they have no reason to suppose, as Gwynne-Vaughan and Williamson do in the case of *Pyronema confluens* (*11*) and *Lachnea scutellata* (*12*), that the first nuclear division in the ascus is a reduction division. The stages in division of prefusion nuclei are too few and too obscure to provide an adequate estimate of chromosomes in haploid nuclei. In those stages observed a single chromatin body appeared to move to each pole of the spindle (fig. 3, *G*, *I*). On the basis of rather meager evidence it appears that there are two unequal chromosomes in diploid nuclei of *C. fimbriata* and that haploid nuclei probably contain a single chromosome. Presumably, if reduction occurs in the ascus, one half of the ascospores and the mycelial growth to which they give origin would contain nuclei possessing a dumbbell-shaped chromosome and the other half would contain nuclei possessing a globular or comma-shaped chromosome. It would be possible, of course, to interpret the bilobed chromatin body as representing two separate chromosomes. In such a case it would be necessary to conclude that the diploid number is three, and that some of the haploid cells contain nuclei with an extra chromosome. In either case the inference would exist that there are two kinds of haploid cells in *C. fimbriata*, differing in the amount of chromatin in their nuclei if not in the number of chromosomes.

As suggested elsewhere, it is probable that no reduction occurs in the ascus of this species and that the chromosome complement of each ascospore is equal. Reduction may occur at the time of ascospore germination or at some later time. Obviously the situation needs to be clarified much further before it can be said that nuclei in *Ceratostomella fimbriata* are not differentiated in respect to sex, and that apparent inequalities in the distribution of chromatin among nuclei may not be in some way related to their behavior.

SECOND NUCLEAR DIVISION

CERATOSTOMELLA MONILIFORMIS

In *Ceratostomella moniliformis* the second division in the ascus resembles in many respects the division of prefusion nuclei. Polar bodies that may correspond to centrosomes are observable in some instances (fig. 4, *M*). Late metaphase stages of this division are particularly obscure, and the observer is disposed to rely upon data from *C. fimbriata* (see below), in which it is shown that no reduction in number of chromatin bodies occurs during the second division. Figure 4, *O* and *Q*, however, shows a difference in the amount of chromatin and in the general appearance of the chromatin mass passing to each pole. The bodies shown probably do not correspond to individual chromosomes.

At an early stage the spindles appear to follow the periphery of the inner vesicle (fig. 4, *P*). Later they appear to traverse the entire breadth of the vesicle (*S*), which by this time has become conspicuously separated from the outer region of the ascus. The curious isolation of the inner vesicle from the outer body of the ascus (*N*, *R*–*Z*, A_1–G_1) is perhaps a characteristic of *Ceratostomella moniliformis*. Mittmann

(*21*) observed a similar phenomenon at a certain stage during ascus formation in *C. fimbriata;* she was uncertain whether or not a wall was present about the vesicle. The present writers have observed the cleavage of the ascus cytoplasm only infrequently in the latter species, while a definite wall is frequently seen in the position corresponding to the periphery of the vesicle in *C. moniliformis.*

No wall appears to surround the spore-producing vesicle in *Ceratostomella moniliformis.* The contracted appearance of the vesicle contents, however, suggests the occurrence of plasmolysis, which in itself may suggest the presence of a membrane. If the phenomenon is actually a result of plasmolysis it nevertheless demonstrates that a material difference, morphological and perhaps physiological, exists between the inner spore-producing mass and the outer much-vacuolated cytoplasm. It seems, however, that the phenomenon, rather than being due to plasmolysis, is similar in nature to the cleavage of the cytoplasm during the formation of ascospores. This is further indicated by the fact that in *C. fimbriata* the inner zone does infrequently develop into a single giant ascospore. (See fig. 7, *L.*)

It is important to note that the contents of the ascus vesicle are not entirely isolated from the outer body of the ascus. The inner mass remains continuous with the external region of the ascus usually at the point where the old nucleolus emerged from the nuclear vesicle (figs. 2, O_1; 4, *T*), and is also connected by slender strands at various points on the periphery corresponding, perhaps, to those observed in the definitive nucleus (fig. 6, *B*). The nucleolus remains permanently in its position at the main attachment of the vesicle contents, and this fact is regarded as evidence that the ascus vesicle (figs. 4, *S–Z*, A_1; 6, *F*) actually corresponds to the nuclear vesicle (fig. 2, A_1, J_1), which has undergone a continuous increase in diameter. The same conclusion was previously reached with respect to *Ceratostomella fimbriata* (*1*), where the ascus vesicle can be shown to possess a definite wall at certain stages.

At the close of the second division in the ascus the daughter nuclei again assume an appearance resembling prefusion nuclei (fig. 4, *T, U*). This stage seems to persist somewhat longer than did the corresponding one following the first division. In figure 4, *T* to *V*, are shown characteristic views of the ascus vesicle at the four-nucleate stage.

Ceratostomella Fimbriata

In *Ceratostomella fimbriata* the second nuclear division in the ascus is similar to the first, there being no appreciable reduction in the size of spindles or in the amount of chromatin. Figures at this stage are less numerous, and details in the distribution of chromatin are not easily observed. It is evident, however, that no reduction in chromosome number occurs at the second division. Each spindle appears as a four- to six-lobed body (fig. 5, *Q, R*), with a two- or three-lobed mass of chromatin passing to either pole (*T*). These resemble in shape and size the chromatin masses most often observed in the first division.

Following the second nuclear division the endogenous ascus wall begins to appear in some of the asci in the position formerly indicated by the outline of the inner mass of denser protoplasm (fig. 5, *S*). While not visible in every instance, it is in many cases distinct and is undoubtedly a true endogenous wall surrounding the inner spore-producing area of the ascus.

THIRD NUCLEAR DIVISION

CERATOSTOMELLA MONILIFORMIS

In *Ceratostomella moniliformis*, owing partly to the reduced size of the various structures, the third division in the ascus is difficult to study. Figure 4, I_1, J_1, shows a stage frequently observed. Metaphase figures are obscure; consequently the division appears to involve only a single chromatin body. The recently reorganized nuclei (fig. 4, X) again resemble those previous to fusion. At a more advanced stage deeply stained bodies, usually crescent-shaped and often bilobed, may represent the reappearance of individual chromosomes at the beginning of ascospore formation (figs. 4, H_1; 6, A, B).

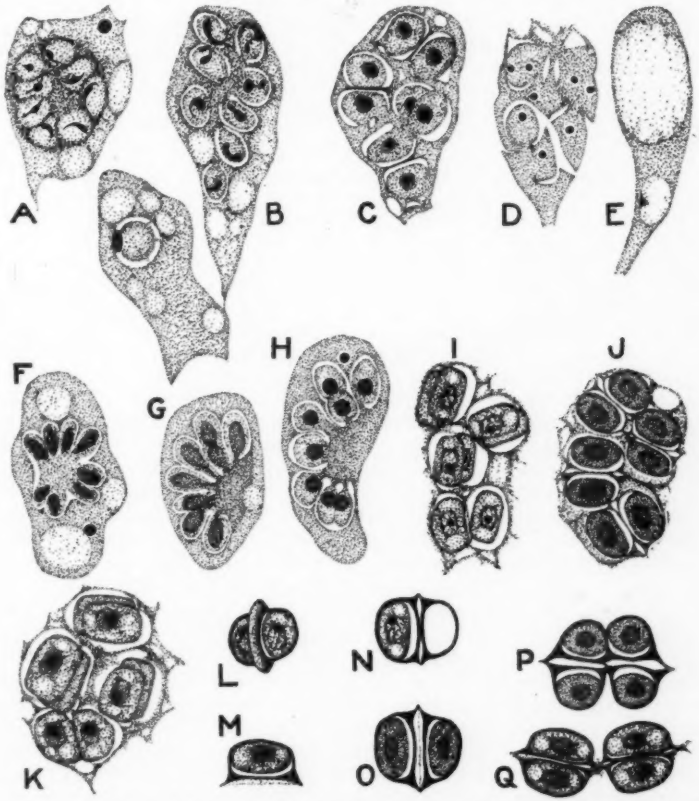

FIGURE 6.—Morphology of the ascus in *Ceratostomella moniliformis*. *A*, An eight-nucleate ascus, with evidence of a membrane surrounding the central mass and with the original nucleolus still visible; *B*, linkage between asci (note the curious shape maintained by chromatic nuclear contents at one stage in spore formation; *C*, an advanced ascus, showing the origin of membranous plates that persist between pairs of ascospores; *D*, an exceptional appearance of ascus cleavage during spore formation; *E*, an abortive ascus, or one from which the spore-producing contents of the vesicle have been removed; *F–H*, typical views of immature asci showing the attachment of the rudimentary spores to a common cytoplasmic base and the formation of spore sheaths by the invagination of the outer region of the ascus; *I–K*, typical mature asci, showing the arrangement of spores in pairs and the absence of a peripheral ascus wall; *L*, an oblique view of a pair of ascospores held together by membranous plates; *M*, a single mature ascospore with membranous attachment; *N*, an ascospore, with a second spore sheath from which the contents have been displaced or in which a spore has failed to develop; *O*, a pair of ascospores held together by membranous plates; *P*, *Q*, groups of four spores held together by membranous attachments and remnants of cytoplasm. All × 3000.

Ceratostomella Fimbriata

In *Ceratostomella fimbriata* there is a slight reduction in the size of spindles and in the amount of chromatin in third-division figures (fig. 7, *C*, *D*). A reduction in number of chromosomes presumably might occur at this stage, yet that is not a necessary conclusion, as is pointed out elsewhere. A brief period of reorganization of daughter nuclei follows the third division. The nuclei frequently have the appearance shown in figure 7, *E*, *H*, where a single deeply stained body in each nucleus resembles in size and shape the chromatin bodies observed at certain stages during the previous nuclear divisions.

A break in the cytoplasm at the limits of the inner cell mass (fig. 7, *E–G*), first described by Mittmann (*21*), seems to be exceptional in this species, although it is a characteristic feature of ascus formation in *Ceratostomella moniliformis* (fig. 4). The occasional occurrence of the break does, however, indicate the vesicular nature of the inner spore-producing mass and is additional evidence of the presence of a membrane surrounding the denser region of the ascus, even though a definite wall is not always to be seen.

SPORE FORMATION AND ORGANIZATION OF MATURE ASCUS

Ceratostomella Moniliformis

In *Ceratostomella moniliformis*, at the first stage in the delimitation of ascospores, the eight nuclei are distributed near the surface of the ball of protoplasm suspended within the ascus vesicle. The chromatic contents of each nucleus appear as a lens-shaped cap with its convex face producing a small wartlike protuberance on the surface of the suspended sphere (fig. 4, *Y*, *Z*). The lens-shaped bodies have somewhat the appearance of polar caps or cones that are observed during spore formation in more familiar Ascomycetes. Polar rays diverging from the apical beak, such as were believed by Harper (*15*) to function in the formation of ascospore walls, seem to be entirely absent in this and related species.

The protuberances on the surface of the sphere of protoplasm suspended in the vesicle are young or rudimentary ascospores. By their continued growth they encroach upon the limits of the vesicle, causing it to become angular in shape (figs. 4, A_1; 6, *F*) and eventually deeply lobed. Each spore rudiment forms a niche in the surrounding wall of protoplasm (fig. 6, *G*, *H*). Numerous indications lead to the conclusion that the eight spores, early in the process of delimitation, radiate from a common center or base (figs. 4, B_1–D_1; 6, *F–H*). This attachment base appears to be a point at which the vesicle contents are continuous with the outer body of the ascus.

As a result of its continued growth each young spore becomes enclosed by a sheath of cytoplasm that originally constituted a portion of the external ascus (fig. 6, *B*, *F–I*). Although the external spore walls appear to be produced by a transformation of the cytoplasm which formerly composed the peripheral region of the ascus, a rudimentary wall may already have existed as a membrane lining the inner face of the epiplasm. The separate origin of the spore wall and the surface membrane of the spore cytoplasm would be somewhat contrary to the observations of Faull (*7*) on *Hydnobolites* and other genera, where the two membranes appeared to originate at

the same point and time. The separation of the spore-producing mass from the epiplasm (as the term is used by Faull) in *Ceratostomella* occurs at approximately the four-nucleate stage, and it may be supposed, of course, that the surface membrane of the inner mass and the membrane that presumably lines the vesicle cavity have a common origin. The two membranes later would appear to become transformed respectively into the surface membrane of the individual spores and into the spore walls.

During advanced stages in spore formation the external portion of the ascus appears to be an inert mass of protoplasm with its outer margin often fading imperceptibly into the unorganized debris of the perithecial cavity. The young ascospores appear to grow into their encasement, yet it is possible that the spore wall is, in part, a result of active growth processes within the cytoplasm. There is no true ascus wall in *Ceratostomella moniliformis*. Close study of the disorganization of the external ascus reveals no stage at which there is an outer membrane of any more definite nature than that which surrounds the numerous vacuoles within the protoplasmic body. The group of mature spores simply fall apart (fig. 6, *I–K*), and remnants of the disorganized cytoplasm remain attached to them after they are discharged from the perithecium.

A characteristic membranous attachment (fig. 6, *M*) that occurs on the ascospores originates from the interspore layers of cytoplasm and seems to be composed of the same substance as the spore walls. The membranous disk-shaped structures are frequently responsible for the spores clinging together in pairs or sometimes in groups of four (fig. 6, *L–Q*).

Ceratostomella Fimbriata

In the initial stage of ascospore formation in *Ceratostomella fimbriata* the eight nuclei emerge near the periphery of the inner cell mass (fig. 7, *G*), and each spore rudiment grows outward and becomes separately enclosed by the surrounding cytoplasm (*H–J*). The young spores extend radially from one or more points of attachment.

The behavior of the endogenous wall during the period of spore formation is variable. As pointed out in a previous publication (1), a wall is not always present in the ascus, in which case the spores mature in a group held together only by remnants of cytoplasm (fig. 7, *O*, *Q*). In other instances a wall is clearly visible following the four-nucleate stage (figs. 5, *U*; 7, *B*), and portions of it are still to be seen after the spores are completely formed (fig. 7, *K*). It appears, therefore, that ascus formation in *Ceratostomella fimbriata* may follow two courses. Where no endogenous wall encloses the spore-producing mass, the cleavage of the young spores may extend into the cytoplasm of the outer ascus (fig. 7, *I*), in which case the spore wall would be principally cytoplasmic in origin. But where an endogenous wall is present, spore cleavage must occur entirely within the vesicle (figs. 5, *V*; 7, *K*), with the vesicle wall being partially used up in the formation of spore walls. The occasional early separation of the central spore-producing region of the ascus from the epiplasm, which is evidenced by a conspicuous cleavage space (fig. 7, *E–G*), suggests that the processes of spore delimitation and membrane formation are fundamentally similar to those described under *C. moniliformis*.

Problems concerning the nature and origin of the spore-producing vesicle and of the vesicle wall in *Ceratostomella* cannot immediately be solved, since there is no precedent for such a structure in any of the Ascomycetes. The opinion has already been expressed that the vesicle wall has its origin from the membrane of the fusion nucleus. Such a view is supported by considerable evidence derived from the study of *C. moniliformis* and is discussed elsewhere. Further information regarding the nature and origin of the endogenous wall in *C. fimbriata* may be had from certain abortive structures that are sometimes observed in the same species. Figure 7, *M*, shows an abortive ascus that appears to contain a single uninucleate spore; figure 7, *R*, seems to represent a similar abortive ascus. Infrequently, as late as the eight-nucleate stage, the central spore-producing mass may become enclosed by a wall of such an indurated appearance that the vesicle seems to be a single giant spore (fig. 7, *L*). It is suggested, therefore, that the delimitation of the vesicle in *Ceratostomella* may be comparable in some respects to the delimitation of a single ascospore.

Mature ascospores of *Ceratostomella fimbriata* possess membranous attachments similar to those of *C. moniliformis*. The attachments originate in a manner similar to the spore walls, namely, from the intervacuolar layers of the cytoplasm (fig. 7, *N*, *P*, *Q*). Spore walls, as represented in figure 7, *O*, for example, at one stage appear as ropy strands or layers of cytoplasm (fig. 7, *P*) and the spore attachments, similar to those shown in figure 6, *M*, are constructed of the same material. These cytoplasmic layers in their turn must have originated from the cytoplasm that formerly enclosed the rudimentary spore cavity (fig. 7, *I*) or from the lining of the vesicle. If spore walls are formed from the cytoplasm of the external ascus, as appears to be the case, and not from the surface layer of the spore rudiments, as is commonly understood to occur in other Ascomycetes, it may be necessary to suppose that ascospore walls in *Ceratostomella* are not entirely equivalent to or homologous with ascospore walls in the more familiar genera. The apparent spore wall in *Ceratostomella* may be a special sheath that encloses both the spore and its true wall; the latter, in these species, may be limited to a surface membrane.

DISCUSSION

Any investigation in the field of cytology that purports to describe something new or something at variance with what is already known of cell or nuclear behavior is apt to meet the familiar query concerning proper fixation. In answer to any possible criticism of this nature it should be said that the several methods used are similar to

EXPLANATORY LEGEND FOR FIGURE 7

A, Cell linkage previous to ascus formation. *B*, A four-nucleate ascus with endogenous wall. *C*, *D*, Asci during the third nuclear division. *E*-*G*, Asci with the spore-producing interior contracted away from the vesicle wall. *H*, An immature ascus, showing the chromatic contents of each spore nucleus organized into distinct bodies. *I*, *J*, Stages in ascospore formation, showing the radial disposition of the young spores. *K*, A nearly mature ascus with a portion of the endogenous vesicle wall still identifiable. *L*, An abortive ascus with the interior developed into a single giant spore. *M*, An abnormal or abortive ascus with a central region that might develop into a giant spore. *N*, A group of ascospores held together by membranous attachments and disorganized cytoplasm of the parent cell. *O*, A well-developed ascus indicating the absence of any peripheral wall. *P*, A pair of ascospores held together by membranous remnants of cytoplasm. *Q*, A fully developed ascus; four of the eight ascospores frequently stain more deeply than the others. *R*, An abnormal or abortive ascus in which a nucleolus has persisted at the margin of the inner cell mass. All × 3000.

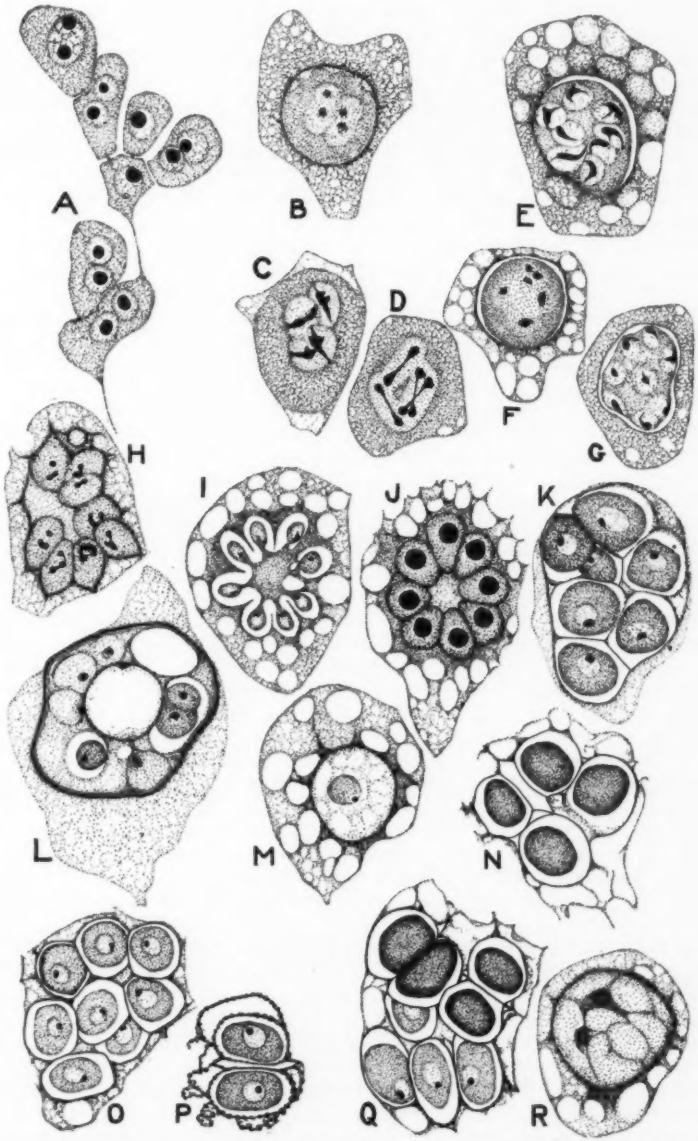

FIGURE 7.—Morphology of the ascus in *Ceratostomella fimbriata*. (For explanatory legend, see opposite page.)

those used successfully by other investigators in the same or related fields and that many of the exceptional phenomena observed in *Ceratostomella*, such as the unwalled condition of the asci and the vesiculate condition of the spore-producing region of the ascus, are clearly discernible in preparations made by the acetocarmine method,[5] where the fruiting bodies are dissected directly in the mounting fluid.

An attempt has been made to describe in some detail the origin and development of an ascus of the so-called deliquescent type. In the course of the discussion considerable emphasis has been placed on the fact that the deliquescent asci of *Ceratostomella* possess no external wall at any stage of their development. The situation at first seems to be unparalleled among other genera of Ascomycetes, but it is possible that those who have had occasion to observe the deliquescent type of ascus have neglected to distinguish between a cell wall, such as occurs on the more familiar type of ascus, and a plasma membrane, which occurs in addition to the external wall. The ascus wall, as it is understood in the case of discomycetous species, for example, is clearly homologous with the hyphal wall present on the filaments on which the asci are characteristically borne. The plasma membrane, on the other hand, is equivalent to the surface layer of cytoplasm such as occurs on any essentially naked or plasmodial mass. The ascus of *Ceratostomella* is of the latter kind. The point at which the external wall is discarded or dissolved away and the point from which the cells of the ascogonium begin to multiply as naked protoplasts can be detected in an early stage of perithecium formation.

Stages in the multiplication of the naked cells previous to ascus formation in *Ceratostomella* have not been adequately described in previous work on the development of the perithecial stage. Crosier cell divisions are of an unusual type and may lead to a better interpretation of the function and nature of the curious device frequently referred to as the "ascus hook." Cell divisions may follow a single cleavage plane, two separate planes of cleavage, two confluent planes of cleavage, or three confluent planes. In particular instances the appearance of a compact group of cells may suggest that simultaneous divisions have occurred in three dimensions. Division of a four-nucleate body into two binucleate portions may follow one of several different courses. The appearance of a crosier may actually be followed by a simple division into two binucleate cells.

All previous investigators, with the exception of Varitchak (*26*), who believed that ascus crosiers occur in *Ceratostomella piceae* Münch, have failed to observe this type of cell division in the genus. Elliott (*6*), Mittmann (*21*), and Andrus and Harter (*1*), all previously overlooked their occurrence in *C. fimbriata*, and the last-named authors were led to question Varitchak's statement concerning *C. piceae* partly owing to the absence of figures showing nuclear division in the supposed crosier. The present observations show that the external form of an ascus hook is not sufficient evidence that the later cell division will be of the crosier type. The position of spindles during the conjugate nuclear division seems to provide the most reliable information as to whether cell division will be direct or indirect. The shape of the cell and the divergent position of spindles, shown in

[5] The so-called "smear" technique frequently employed by cytogeneticists.

figure 2, *J*, are very characteristic, especially in *C. moniliformis*. It seems necessary to suppose that conditions of polarity within the cell determine both the shape of the cell and the position of spindles at the time of nuclear division but that the two conditions may not always be correlated.

The vesiculate structure of the spore-producing region of the ascus in *Ceratostomella moniliformis* and *C. fimbriata* seems to be quite without parallel in other fungi. Mittmann (*21*) first described the break in the cytoplasm bounding the inner spore-producing region in *C. fimbriata*. She was uncertain whether or not a membrane or wall was present. Andrus and Harter (*1*) did not observe the cleavage of the cytoplasm in *C. fimbriata*, but frequently observed the presence of an endogenous wall surrounding the spore-producing area. They believed that the position of the wall corresponded to the periphery of the original (definitive) nuclear vesicle and concluded that the nuclear membrane, if such had existed, had been transformed into the endogenous ascus wall. In the present investigation the break in the cytoplasm of the ascus is found to occur in some asci and the existence of an endogenous wall is more clearly demonstrated. In *C. moniliformis*, on the other hand, the break in the cytoplasm surrounding the spore-producing region is highly characteristic and there appears to be no endogenous wall, except such as might be suggested by the appearance of plasmolysis (fig. 4, *S–Z*).

The remarkable structure of the spore-producing area of the ascus in *Ceratostomella moniliformis* and *C. fimbriata* does not seem to be merely an exaggeration of the cytoplasmic condensation so frequently observed in more conventional Ascomycetes. To be sure the vacuolization of the outer region of the ascus and the denser, more homogeneous appearance of the inner region correspond to the condition in other species, but in *C. moniliformis* and *C. fimbriata* nuclear divisions and spore formation seem to take place within a true vesicle that is variously separated from the outer region of the ascus by a true wall, by a conspicuous cleavage of the cytoplasm, or by an abrupt and regular contour.

The fact that the cleavage of the ascus cytoplasm in *Ceratostomella moniliformis* sometimes occurs as early as the first nuclear division is considered to be especially strong evidence that the margin of the spore-producing vesicle actually corresponds to the margin of the original fusion nucleus, since the first spindle, at least, is intranuclear. The position of the nucleolus in relation to the nuclear vesicle during the first division and in relation to the ascus vesicle at a later stage is further evidence that the original nuclear vesicle is continuous with or is transformed into the spore-producing vesicle. These evidences would seem to be very conclusive were it not for the fact that there is ordinarily a brief period following the first division during which the outline of the vesicle is very faint or, in particular asci, not at all apparent. It may be difficult to determine whether the nuclear membrane actually becomes transformed into the membrane of the spore-producing vesicle, but such a view seems to be a logical one.

The occurrence of successive nuclear divisions within an original nuclear vesicle is not without precedent, for it has been described frequently in other species of fungi and in algae. The regeneration of an entire cellular unit from a nucleus or from chromatic portions of a nucleus also is not without precedent if the opinions of Komocki

(*16*) in regard to the erythrocytes of the salamander *Batrachoseps attenuatus* Esch. are accepted. The formation of an endogenous wall or walled inclusion within a cytoplasmic body certainly is not without precedent. Nevertheless, the statement concerning the "transformation of a nuclear membrane into an ascus wall" is astonishing and much further study is necessary before it can be accepted fully. Doubts such as were expressed by Gwynne-Vaughan and Williamson (*13*) regarding earlier work on *Ceratostomella fimbriata* (*1*) are not surprising.

The question of the nature of the spore-producing vesicle in asci of *Ceratostomella fimbriata* and *C. moniliformis*, and perhaps other members of the genus, is quite as important as that concerning the origin of the vesicle membrane. It has already been indicated that the spore-producing region of the cytoplasm in asci of more familiar genera often appears condensed in contrast to the vacuolated peripheral or sometimes basal region of the ascus, but the denser portion does not appear to be vesiculate. The vesiculate condition appears to be lacking also in *C. piceae* (*26*), which belongs to the group of non-endoconidial species. Since the outer region of the ascus in *Ceratostomella* is a naked and sometimes shapeless mass, the view that the vesicle membrane represents or substitutes for an ascus wall would seem to be an obvious one. As a matter of fact, some asci appear to have no wall either inside or out, the intervacuolar layers of cytoplasm representing the nearest approach to a membranous structure.

The interpretation now suggested is that the spore-producing vesicle corresponds to a single giant ascospore. In *Ceratostomella fimbriata* the "giant spore" first appears as a definitely walled structure; in *C. moniliformis* (fig. 4, *K*, *N*) it is formed by a process of cytoplasmic cleavage that appears to be very similar to the cleavage by which ascospores are formed in more familiar genera of the Ascomycetes. The eight-nucleate "spore" seems sometimes to persist as a single abortive ascospore (fig. 7, *L*), but characteristically the vesiculate body either gives rise to eight protuberances (fig. 4, *Z*) that at first remain attached to a common base (figs. 4, B_1, D_1; 6, *F*; 7, *I*) but later appear as eight separate ascospores (figs. 6, *B*; 7, *O*) or becomes separated into eight spores, apparently by the usual processes of ascospore delimitation.

Whether or not the above interpretation of the ascus vesicle in *Ceratostomella* is correct, it is still evident that the process of ascospore formation differs from that commonly understood to occur in other Ascomycetes. Although the cleavage of the cytoplasm about the spores may in some instances be comparable to that observed in other species, the manner of outgrowth of spores, i. e., their whorled arrangement and their attachment to a common base, does not seem to conform to any previous description of ascospore formation, and the clearly vesiculate condition of the spore-producing region is of course unique.

The absence of spindle fibers and polar rays, such as were believed by Harper (*15*), Schultz (*25*), and others to function actively in the process of spore-wall formation, is further evidence that the process of ascospore delimitation in *Ceratostomella* may differ fundamentally from that commonly observed to occur. If it may be conceived that the differentiation of the spore-producing vesicle in *Ceratostomella* corresponds to the delimitation of a single ascospore, then the process

would seem to conform more nearly with the process of ascospore formation described by Faull (*7*), especially in regard to membrane and spore-wall formation.

The question of where the reduction division occurs may turn out to be an especially significant one in respect to the genus *Ceratostomella*. Buisman (*2*) observed that single-conidium cultures of *C. fagi* Loos fall into two sexual groups and that ascospores seem to be bisexual. Single-ascospore cultures produced perithecia, but cultures from single conidia did not. Mating of single-conidium cultures resulted in the formation of perithecia. Buisman suggested that sex segregation may occur at the time of ascospore germination. A difference in the behavior of cultures derived from ascospores and from conidia had been observed previously by McCallum (*19*), Münch (*22*), and Loos (*18*) in several species of *Ceratostomella*. Leach (*17*) also has observed recently a difference in the ability of *C. ips* Rumbold to produce perithecia in cultures of conidium and ascospore origin, but neither type of reproductive cell appeared to be wholly bisexual or wholly unisexual. These curious facts suggest that a segregation occurs at the time of ascospore germination or at some time during the period of mycelial growth or conidium formation. It is probable, although perhaps it is not a necessary conclusion, that sex segregation occurs at the time of, or is accompanied by, reduction in the number of chromosomes.

Ceratostomella moniliformis and *C. fimbriata* appear to be homothallic. Single ascospore cultures of both species produce perithecia. Out of 32 single-conidium cultures of *C. fimbriata*, only 4 failed to produce perithecia. At present it is unknown whether the four cultures represent sex segregates or some other type of heritable sterility, such, for example, as frequently appear as sectors in plate colonies of the same species. The homothallic condition of the two species seems to support the earlier observation that there is no reduction in number of chromosomes in the ascus, and that the two chromosomes observed actually represent the diploid number. On the other hand it may be a mistake to assume that a single-ascospore culture is diploid merely because it is able to produce perithecia.

The view of Gwynne-Vaughan and her students that a double reduction occurs in the ascus is made necessary by their belief that a double nuclear fusion occurs in the ontogeny of certain species. The occurrence of nuclear fusion in the ascogonium, however, has been to a considerable extent discredited, especially by the recent work of Raymond (*23*), who observed that fusions of the type described by Fraser (*8*) and Fraser and Brooks (*9*) in *Lachnea stercorea* and by Gwynne-Vaughan and Williamson (*11*) in *Pyronema confluens* occur also in wholly vegetative cells.

Conclusions in regard to sex segregation and chromosome reduction in *Ceratostomella* should be deferred for the present. It is conceivable that reduction as well as sex segregation might occur at various points in the life cycle. Many fundamental facts regarding cytological behavior and sex segregation in fungi still remain to be discovered. Certainly it cannot be said that knowledge of the life history and cytology of *C. moniliformis* and *C. fimbriata* is in any way complete. It may yet be possible to determine whether nuclei at many different stages are haploid or diploid, and such information would be of great value in interpreting life histories.

SUMMARY

Stages in the multiplication of unwalled cells previous to ascus formation in *Ceratostomella moniliformis* and *C. fimbriata* include the crosier type of cell division as well as several other types of direct and indirect cleavage. The position of spindles at the time of nuclear division is believed to provide the most reliable indication as to whether cell division will be direct or indirect. Cell divisions are accompanied by significant changes in cell shape. A structure resembling a centrosome in behavior is present as a satellite of nucleoli during the prefusion stage.

Nuclear fusion in the ascus is followed by three successive nuclear divisions, all of which are alike except in the size of the various structures. The axis of the spindle is a simple rod without evidence of polar bodies or astral rays. Two and later four chromatin bodies appear on the axis of the spindle, and two bodies move to each pole. The chromosome number in each species is interpreted to be 2 (diploid). No reduction occurs in the ascus.

Following the nuclear divisions the spore-producing region of the ascus is differentiated as a single distinct vesicle. The vesiculate condition in *Ceratostomella moniliformis* is made evident by the presence of a cleavage space surrounding the spore-producing region, while in *C. fimbriata* a definite endogenous wall frequently encloses the vesicle. Data are presented which tend to show that the membrane of the spore-producing vesicle is continuous with or derived from the membrane of the fusion nucleus.

The eight ascospores in *Ceratostomella moniliformis* originate as protuberances on the surface of the sphere of protoplasm contained in the spore-producing vesicle. By continued growth each spore becomes enclosed by a wall of cytoplasm that originally composed a part of the external ascus. The process of spore formation in *C. fimbriata* is frequently modified to an extent imposed by the presence of a wall enclosing the spore-producing region of the ascus. In both species the immature spores appear to be attached to a common base which is usually lateral to the longer dimension of the ascus.

The mature ascus consists of a compact group of spores to which are attached remnants of inert cytoplasm. Deliquescence of the ascus involves a disorganization of the peripheral layer of cytoplasm but does not appear to involve any process of wall dissolution in *Ceratostomella moniliformis* and *C. fimbriata*, since the asci possess no external wall. Mature ascospores possess disk-shaped membranous attachments that appear to originate in a manner similar to spore walls and often hold the spores together in pairs or in groups of four.

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EFFECT OF STAND DENSITY ON MORTALITY AND GROWTH OF LOBLOLLY PINE [1]

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INTRODUCTION

Very few long-time records of the growth and development of individual forest stands are available. Some of the earliest permanent sample plots in this country on which periodic measurements of trees have been made were established by the Forest Service in 1906 in old-field stands of loblolly pine (*Pinus taeda* L.) on the Eastern Shore of Maryland. Though the data from these plots are inadequate according to present-day standards, analyses of them should be helpful to foresters who are studying ways and means of sampling to give more satisfactory and dependable results.

The present study of growth and mortality is the second analysis of the Maryland loblolly pine data. The first dealt with diameter distributions (*6*).[3]

DESCRIPTION OF SAMPLE PLOTS

Since the Federal Government owned no forest land on the Eastern Shore, the plots were established on private land in Worcester County, with the consent of the owners and their promise to allow the plots to remain undisturbed. In the ensuing years, however, many of the plots have been disturbed in one way or another, so that at the time of a remeasurement, if evidences of other than natural disturbances were noted upon certain plots, those plots were discontinued from the date of the previous measurement. Thus the number of plots still remaining in a state suitable to the purposes for which the experiment was originally started has been considerably reduced.

The plots varied in size from 0.1 to 0.5 acre. All trees larger than 2.5 inches d. b. h.[4] were numbered and their diameters measured. Since the range of ages in 1906 was from 3 to 42 years, many plots contained trees smaller than 2.5 inches in diameter. These, 0.5 to 2.4 inches in diameter, were grouped as "reproduction", and only those subsequently reaching 2.5 inches were numbered at the time of the following remeasurement. Height measurements were made of several trees on each plot in order that height-over-diameter curves might be drawn. The measurements were insufficient, however, to make possible a separate curve for each plot.

Certain of the plots were laid out in pairs of which one was thinned in 1906 and the adjoining one left intact. The number, size, and volume of the trees removed were recorded. The thinnings were extremely light and were not periodically repeated in the following years. Other plots were laid out in young stands and located at various distances from the adjacent old timber areas from which they were seeded. These plots varied considerably in stand density.

[1] Received for publication Aug. 7, 1936; issued February, 1937.
[2] Maintained at Philadelphia, Pa., in cooperation with the University of Pennsylvania.
[3] Reference is made by number (italic) to Literature Cited, p. 58.
[4] Diameter breast high—4.5 feet above average ground level.

Remeasurements were made in 1910, 1915, 1920, 1926, and 1930. Thus there is a 25-year record of progress for those plots that are still in existence and shorter records for those that have been discontinued. In this study 44 plots have been used in part.

In general the plots are in old-field stands typical of the Eastern Shore. The range of sites is not quite so extensive as that for the whole region as found by Cope (*2*), but it is believed that the conclusions reached will apply to most if not all stands in this region. Also, any fundamental relations found to exist in these data may well find application in other forest stands.

METHODS AND RESULTS

BASAL AREA AND VARIABLES

Basal area was chosen as the most suitable unit for expressing growth because of the ease and accuracy with which it may be computed and its importance in volume comparisons to be made later. The next step was to determine the major factors influencing growth. Although the number of variables with which growth in even-aged stands may be correlated is large, Bruce and Schumacher (*1*) group them into stand density, age, and site index, the latter representing the combined effect of soil and climatic factors. The number of variables is thus limited to three. (Stand basal area at the beginning of the period was used later in the study as a fourth variable. It is, of course, highly correlated with density.)

The age of each stand was known; the range of ages of the trees in any one stand was so narrow that its determination presented no problem. There remained the necessity of establishing a method of measuring site index and stand density. Site index was read from Forest Service site index curves for loblolly pine (*7*).

The determination of stand density proved to be somewhat more of a problem. Reineke (*5*) has presented a method based on the correlation existing between number of trees per acre and the average diameter of the stand. He has demonstrated that this relationship exists in even-aged stands for many species, and that it can be expressed mathematically by the equation:

$$\log N = -1.605 \log D + K$$

in which N is the number of trees per acre, D is their average diameter (by basal area), and K is a constant varying with species.

Reineke's principle has been used in establishing the density of the plots in this study, but the method of obtaining the final equation has differed. He plotted the number of trees per acre over average diameter for a number of stands of selected species on logarithmic cross-section paper, the data being contemporaneous measurements of stands of various ages and site qualities. He then adjusted the above reference equation to the maximum plotted points. The density of any stand was then computed as a percentage of this maximum. In the present work there was a series of plots upon which periodic measurements had been taken. Upon testing for the correlation between number of trees per acre and their average diameter, using logarithmic units, it was found that each stand, once established, showed throughout the rest of its life a trend similar to that reported by Reineke. Thus for each stand it was possible to obtain an equation representing its average density of stocking. By averaging

these equations, an equation representing the average density of stocking for all plots was derived. Each equation was of the form:

$$\log N = b\ (\log x) + K$$

in which b is the regression coefficient and K is a constant. Averaging was accomplished by adding and averaging the b's and K's. The average density equation obtained is:

$$\log N = -1.603 \log D + 4.075$$

in which N is the number of trees per acre, D their average diameter breast high (by basal area), and 4.075 the constant. The density of each plot was then referred to as a percentage of this average (not maximum) density.

Where consecutive measurements of permanent sample plots are available the authors believe that this average density line may be obtained with much fewer measurements than where contemporaneous measurements in different stands must be used. The very significant correlation existing between number of trees per acre and their average diameter is shown so clearly throughout the life of a single stand, once the stand has become established, as to leave no room for doubt as to its existence.

It might be well at this point to explain that in this work a stand was considered to have become established when all of the trees were large enough to come within the range of the measurements. Obviously, plots containing trees smaller than those actually measured (lower limit 2.5 inches d. b. h., as previously explained), do not give a true picture of the relation between number of trees per acre and their average diameter, since all trees were not included in the measurements. Had all trees been measured, the same regression might well have appeared with the beginning of the stand.

From the above equation the density of each stand (plot) was computed. The number of trees per acre required to attain certain percentages of average density is shown in table 1.

TABLE 1.—*Number of trees per acre for various stand densities*

Percentage of average density	Number of trees per acre by average stand diameter of—[1]																
	3.0 inches	3.5 inches	4.0 inches	4.5 inches	5.0 inches	5.5 inches	6.0 inches	6.5 inches	7.0 inches	7.5 inches	8.0 inches	8.5 inches	9.0 inches	9.5 inches	10.0 inches	10.5 inches	11.0 inches
60	1,225	958	773	640	541	464	404	355	315	282	254	231	211	193	178	164	153
65	1,327	1,037	837	694	586	502	437	385	341	306	276	250	228	209	193	178	166
70	1,429	1,117	902	747	631	541	471	414	368	329	297	270	246	225	208	192	178
75	1,532	1,197	966	800	676	580	505	444	394	352	318	289	263	242	223	206	191
80	1,634	1,277	1,030	854	722	618	538	474	420	376	339	308	281	258	238	219	204
85	1,736	1,357	1,095	907	767	657	572	503	446	400	360	327	298	274	252	233	217
90	1,838	1,436	1,159	960	812	696	606	533	472	423	382	346	316	290	267	247	230
95	1,940	1,516	1,224	1,014	857	734	639	562	499	446	403	366	333	306	282	260	242
100	2,042	1,596	1,288	1,067	902	773	673	592	525	470	424	385	351	322	297	274	255
105	2,144	1,676	1,352	1,120	947	812	707	622	551	494	445	404	369	338	312	288	268
110	2,246	1,756	1,417	1,174	992	850	740	651	578	517	466	424	386	354	327	301	280
115	2,348	1,835	1,481	1,227	1,037	889	774	681	604	540	488	443	404	370	342	315	293
120	2,450	1,915	1,546	1,280	1,082	928	808	710	630	564	509	462	421	386	356	329	306
125	2,552	1,995	1,610	1,334	1,128	966	841	740	656	588	530	481	439	402	371	342	319
130	2,655	2,075	1,674	1,387	1,173	1,005	875	770	682	611	551	500	456	419	386	356	332
135	2,757	2,155	1,739	1,440	1,218	1,044	909	799	709	634	572	520	474	435	401	370	344
140	2,859	2,234	1,803	1,494	1,263	1,082	942	829	735	658	594	539	491	451	416	384	357

[1] Based on equation: log number of trees per acre=4.075−1.603 log average diameter breast high.

Attention was next directed to a choice of measures for expressing growth in basal area. The alternatives were absolute units (in square feet) and percentages—ratios between the increment and the amount previously present. Percentages were chosen because they may be likened to rates of return upon a stated amount of capital stock, and are therefore easily understood and transferable into absolute units with little difficulty. Summations of the individual tree measurements gave the amount of basal area present at 5-year intervals. By taking the difference in the amount of basal area present at the beginning and end of a 5-year period, the net growth was obtained for that period. It occasionally happened that the measurement at the end of the 5 years disclosed a smaller amount of basal area than had been there at the first measurement, indicating that during the 5 years more basal area had dropped out through the death of trees than had been gained by growth on those remaining. In this case the net growth for the period was recorded as a negative amount.

The net-growth figures for each 5-year period were then transferred into percentages, giving the relative rates of growth. The method used was to ascertain the natural logarithm of the amount of basal area present at the beginning and end of the 5-year period and subtract one from the other, multiplying the result by 100 to obtain a percentage value, as is done by Fisher (*4*).

This is an application of the compound interest formula presented by Dull (*3*)

$$A = Pe^{rn}$$

in which A is the amount of any principal (P) after n years compounded at r rate of interest, and e is the base of the natural system of logarithms.

Having measures of stand density, stand basal area, site index, age, and rate of growth, for each stand, the following correlations were then computed:

TWO-VARIABLE CORRELATIONS Variables correlated:	*Correlation coefficient*
Rate of growth (5-year period) and site index	−0. 16034
Rate of growth (5-year period) and age	−. 32234
Rate of growth (5-year period) and density	−. 44442
Rate of growth (5-year period) and basal area	−. 48683
Basal area per acre (beginning of period) and site index	+. 24718
Basal area per acre (beginning of period) and age	+. 42366
Basal area per acre (beginning of period) and density	+. 84151
Density (beginning of period) and site index	+. 20937
Density (beginning of period) and age	+. 01205
Site index and age	−. 22794

THREE-VARIABLE CORRELATIONS Variables correlated:	
Rate of growth (5-year period) with density and basal area	+. 49107
Rate of growth (5-year period) with density and age	+. 54590
Rate of growth (5-year period) with density and site index	+. 44971
Rate of growth (5-year period) with basal area and age	+. 50352
Rate of growth (5-year period) with basal area and site index	+. 48858

FOUR-VARIABLE CORRELATIONS	*Correlation coefficient*
Variables correlated:	
Rate of growth (5-year period) with density, site index, and age	+0.56553
Rate of growth (5-year period) with basal area, site index, and age	+.51269
Rate of growth (5-year period) with density, basal area, and age	+.54841

FIVE-VARIABLE CORRELATION	
Variables correlated:	
Rate of growth (5-year period) with density, basal area, site index, and age	+.58426

Since 108 cases were used in the correlations, any coefficient larger than 0.24056 (2.5 times the error) is considered significant. On this basis, six of the two-variable correlations are significant. It is well to call attention to the fact that rate of growth is not significantly correlated with site index. Site is reflected in the amount of wood material present but not in the rate of increase.

Although the multiple correlations show some improvement over the simple ones, the practical value of including so many variables is doubtful. The five-variable correlation is very little better than the four, and the four-variable correlations show very little improvement over the three. Because the simple correlations show density and basal area originally present to be the most important factors, and because basal area is significantly correlated with age and site, while density is not, it is felt that the three-variable correlation of growth with density and basal area should reflect all factors. This is borne out by the errors of estimate of the several regression equations. The use of this correlation eliminates the necessity of measuring age and site index, which simplifies application to actual field problems; then, too, foresters are accustomed to thinking in terms of basal area. While stand density may be the controlling factor, the inclusion of basal area as a variable permits a more comprehensive table.

With the elimination of site index as a variable it was possible to include seven more stands in which insufficient height measurements had prevented site classification. The correlations thus strengthened are presented in table 2, together with similar correlations for 10- and 15-year periods and the standard errors.

TABLE 2.—*Growth correlations for 5-, 10-, and 15-year periods* [1]

Relative rate of growth (percent of basal area) correlated with—	For 5-year period [2]		For 10-year period [3]		For 15-year period [4]	
	Correlation coefficient	Error	Correlation coefficient	Error	Correlation coefficient	Error
(1) Percent of average density at beginning of period	−0.465	±0.073	−0.622	±0.072	−0.694	±0.083
(2) Square feet of basal area per acre present at beginning of period	−.494	±.071	−.688	±.062	−.749	±.070
(3) Percent of average density and square feet of basal area per acre present at beginning of period	+.503	±.071	+.691	±.062	+.752	±.072

[1] The increase in correlation with age is probably due to built-up internal correlations. Growth for a particular 5-year period is often included twice in the 10-year correlations, as the upper 5 years of one 10-year period and as the lower 5 years of another.

[2] 115 cases.

[3] 73 cases.

[4] 39 cases.

Regression equations, by means of which future rates of growth for periods up to 15 years [5] may be estimated within the limits shown, were then computed. These equations and their standard errors of estimate appear in table 3.

TABLE 3.—*Regression equations for predicting relative rate of growth*

Period	Regression equation [1]	Standard error of estimate	Number of cases
5 years	$X_1 = -0.1001 X_2 - 0.1224 X_3 + 34.9$	7.4	115
10 years	$X_1 = -.1090 X_2 - .3025 X_3 + 67.9$	8.9	73
15 years	$X_1 = -.1420 X_2 - .3970 X_3 + 87.8$	10.5	39

[1] X_1=relative rate of growth in percent basal area. X_2=percentage of average density at beginning of period. X_3=square feet of basal area per acre present at beginning of period.

In order to place the growth-predicting equations in a more usable form, alinement charts were constructed from which the values in table 4 were read. Given the percentage of average density of a stand, as interpolated from table 1, and the basal area present, the estimated rate of future growth may be read from this table.

TABLE 4.—*Net basal-area growth in 5, 10, and 15 years, in percent of basal area per acre at beginning of period, for stands of different densities* [1]

5 YEARS

Percentage of average density	Growth (percent) by basal area which at beginning of period was—															
	60 square feet	70 square feet	80 square feet	90 square feet	100 square feet	110 square feet	120 square feet	130 square feet	140 square feet	150 square feet	160 square feet	170 square feet	180 square feet	190 square feet	200 square feet	
60	21.6	20.3	19.1	17.8	16.7	15.4	14.2	13.0	11.8	10.6						
65	21.0	19.8	18.6	17.4	16.2	15.0	13.7	12.5	11.3	10.1						
70	20.5	19.3	18.1	16.9	15.6	14.5	13.3	12.0	10.8	9.6	8.4	7.2				
75	20.0	18.8	17.6	16.4	15.2	13.9	12.7	11.5	10.2	9.1	7.8	6.6				
80	19.6	18.3	17.1	15.9	14.7	13.4	12.3	11.0	9.8	8.6	7.4	6.2	4.9			
85	19.0	17.9	16.6	15.4	14.2	12.9	11.7	10.5	9.3	8.1	6.8	5.7	4.4			
90	18.5	17.3	16.1	14.8	13.6	12.5	11.3	10.0	8.8	7.6	6.4	5.1	4.0			
95	18.0	16.7	15.6	14.4	13.2	11.9	10.7	9.5	8.3	7.1	5.8	4.6	3.5			
100	17.6	16.3	15.1	13.8	12.6	11.4	10.2	9.0	7.8	6.6	5.3	4.2	2.8			
105		15.8	14.6	13.4	12.2	11.0	9.7	8.5	7.3	6.1	4.8	3.7	2.3			
110		15.3	14.1	12.8	11.6	10.3	9.2	8.0	6.8	5.6	4.3	3.1	1.8			
115			13.6	12.3	11.2	9.9	8.7	7.5	6.3	5.1	3.8	2.6	1.4	+0.2		
120			13.1	11.8	10.6	9.4	8.2	7.0	5.7	4.6	3.3	2.1	.8	−.3	−1.5	
125			12.6	11.4	10.1	8.9	7.7	6.5	5.2	4.1	2.9	1.6	+.4	−.8	−2.0	
130				10.8	9.6	8.5	7.2	6.0	4.7	3.6	2.3	1.1	−.1	−1.2	−2.5	
135				10.3	9.2	8.0	6.7	5.5	4.2	3.1	1.7	.6	−.6	−1.8	−3.0	
140					8.6	7.4	6.2	5.0	3.7	2.6	1.3	.1	−1.1	−2.3	−3.5	

[1] Block indicates extent of basic data.

[5] At the time these analyses were made there were insufficient measurements to permit estimates of rates of growth beyond 15 years.

TABLE 4.—*Net basal-area growth in 5, 10, and 15 years, in percent of basal area per acre at beginning of period, for stands of different densities*—Continued

10 YEARS

Percentage of average density	Growth (percent) by basal area which at beginning of period was—															
	60 square feet	70 square feet	80 square feet	90 square feet	100 square feet	110 square feet	120 square feet	130 square feet	140 square feet	150 square feet	160 square feet	170 square feet	180 square feet	190 square feet	200 square feet
60	42.9	40.0	36.9	33.9	30.9	28.0	24.9	21.8	18.9	15.9					
65	42.4	39.5	36.5	33.5	30.5	27.6	24.5	21.4	18.5	15.5					
70	42.1	39.2	36.2	33.1	30.1	27.2	24.1	21.0	18.1	15.0	12.0	9.0			
75	41.8	38.8	35.8	32.6	29.7	26.7	23.7	20.7	17.7	14.7	11.7	8.7			
80	41.4	38.4	35.4	32.2	29.3	26.3	23.3	20.3	17.2	14.2	11.2	8.2	5.2		
85	41.0	38.0	35.0	31.9	28.9	25.9	22.9	19.9	16.8	13.8	10.8	7.8	4.8		
90	40.7	37.7	34.6	31.6	28.6	25.5	22.4	19.5	16.5	13.5	10.4	7.3	4.3		
95	40.2	37.1	34.1	31.1	28.2	25.1	22.0	19.1	16.0	13.0	10.0	6.9	3.9		
100	39.8	36.8	33.8	30.8	27.8	24.8	21.7	18.7	15.6	12.5	9.6	6.6	3.7		
105	.....	36.4	33.3	30.3	27.3	24.3	21.2	18.2	15.2	12.1	9.2	6.1	3.2		
110	.....	35.9	32.9	30.0	26.9	23.9	20.8	17.8	14.9	11.8	8.9	5.7	2.7		
115	.....	.....	32.5	29.6	26.5	23.5	20.4	17.4	14.4	11.4	8.5	5.3	2.3	−0.5	
120	.....	.....	32.1	29.1	26.1	23.1	20.1	17.0	14.1	11.0	8.0	5.0	1.9	−1.0	−4.1
125	.....	.....	31.8	28.9	25.8	22.6	19.6	16.6	13.6	10.5	7.6	4.6	1.5	−1.3	−4.5
130	.....	.....	.....	28.4	25.3	22.3	19.2	16.1	13.2	10.2	7.1	4.1	1.1	−1.7	−4.9
135	.....	.....	.....	28.0	25.0	21.9	18.9	15.7	12.6	9.8	6.7	3.8	.7	−2.3	−5.3
140	.....	.....	.....	.....	24.6	21.4	18.5	15.3	12.3	9.4	6.3	3.4	.3	−2.7	−5.7

15 YEARS

60	51.5	48.6	45.6	42.7	39.7	36.6	33.6	30.6	27.8	24.9					
65	51.0	48.1	45.1	42.1	39.1	36.0	33.0	30.1	27.2	24.3					
70	50.5	47.6	44.6	41.5	38.5	35.6	32.5	29.7	26.7	23.8	20.8	17.8			
75	50.0	47.0	44.0	41.0	38.0	35.0	32.0	29.1	26.0	23.1	20.2	17.1			
80	49.5	46.5	43.5	40.5	37.5	34.5	31.4	28.6	25.7	22.8	19.8	16.7	13.7		
85	48.9	45.9	42.9	40.0	37.0	33.9	30.9	28.0	25.1	22.1	19.1	16.0	13.0		
90	48.5	45.4	42.5	39.5	36.3	33.4	30.5	27.5	24.6	21.7	18.6	15.6	12.7		
95	47.8	44.9	41.9	38.9	35.9	32.9	30.0	27.0	24.1	21.0	18.0	15.1	12.0		
100	47.3	44.3	41.3	38.3	35.3	32.3	29.4	26.3	23.4	20.5	17.5	14.5	11.6		
105	.....	43.8	40.7	37.8	34.8	31.8	28.9	25.9	22.9	20.0	17.0	14.0	10.9		
110	.....	43.2	40.2	37.2	34.2	31.2	28.2	25.3	22.3	19.5	16.4	13.3	10.5		
115	.....	.....	39.7	36.5	33.6	30.6	27.7	24.9	21.9	18.9	15.9	12.9	10.0	7.0	
120	.....	.....	39.2	36.1	33.1	30.2	27.3	24.4	21.3	18.3	15.4	12.4	9.5	6.4	3.5
125	.....	.....	38.6	35.6	32.6	29.7	26.6	23.7	20.7	17.8	14.9	11.9	8.9	5.9	3.0
130	.....	.....	.....	35.1	32.1	29.2	26.1	23.1	20.3	17.3	14.2	11.2	8.2	5.7	2.5
135	.....	.....	.....	34.6	31.5	28.5	25.7	22.6	19.7	16.7	13.7	10.8	7.8	5.0	2.0
140	.....	.....	.....	.....	30.9	28.1	25.2	22.1	19.2	16.2	13.1	10.3	7.2	4.6	1.3

Since growth was computed with the compound-interest formula, the values in table 4 are average compound rates for the periods indicated. A growth of 15 percent for a 5-year period means an average compound annual growth rate of 3 percent per year. For 1 year the rate may be 4, for another 2, but for the 5-year period the average is 3.

APPLICATION OF RESULTS TO THINNINGS

Up to this point the study seemed to lead to the conclusion that the subsequent rate of growth of a stand was always correlated with the density and basal area present at any particular time. This would indicate that a change in either or both of these two factors would result in a change in subsequent rate of growth.[6]

[6] In first computing these correlations, plots upon which thinnings had been made were segregated from those left intact. Similar regressions existed in each set, so all plots were thereafter combined.

From the standpoint of application to thinnings the next problem to be solved, therefore, was how much to change the density and basal area at any one time to give the best resultant rate of growth. According to the measures of density described earlier, any reduction in number of trees per acre, such as would be accomplished in a thinning, would result in a change of density. That the amount of basal area present would be changed is apparent. Thus any indication of how much to change these two factors would be at the same time an indication of how much to thin.

At various times during the analysis of the material certain facts seemed to indicate that a significant correlation might exist between the rate of growth during any period and the amount which died during the same period. Adding this third factor—amount which died, or mortality, during the period—to the multiple correlation found previously, the correlation between rate of growth and density, basal area at the beginning, and amount which died, was found to increase to the high figure of 0.835 ± 0.029 (table 5).

TABLE 5.—*Growth correlations for 5-year period, using amount which died as one of the independent variables*

Relative rate of growth (percentage of total basal area) correlated with—	Correlation coefficient	Standard error of correlation coefficient
Percentage of basal area in square feet per acre dying during period	−0.602	±0.059
Percentage of basal area in square feet per acre dying during period, percentage of average density at beginning of period, and square feet of basal area present at beginning of period	+.835	±.029

This mathematical process led to the familiar conclusion that if the probable mortality in a stand during the coming years could be estimated and the doomed trees removed in advance, the wood might be salvaged and the rate of growth accelerated.

Death in a stand of timber is the result of fierce competition between the trees for the essentials of life, and this struggle has a retarding effect upon rate of growth as long as those trees that will eventually die still live. Obviously, therefore, when this competition is removed, the rate of growth on the trees remaining can increase. Mortality, then, is an expression of the number of trees in the way, acting as a retarding agent upon those better fitted to form the mature stand, and should form a guide as to the number which should be removed. Recalling the definition of net growth—amount that grew in excess of amount that died—it can be seen that if there were no deaths occurring in the stand, gross and net increase would be identical, and the stand would return the maximum rate of interest on the capital, consistent with the other governing factors—density and amount of basal area. If, in addition, a market were available for the material removed in thinning, the loss would have been eliminated completely from the stand. This is the generally accepted theory concerning thinnings, now established on a sounder statistical basis.

To use future mortality as a guide as to how much to remove in a thinning, a method of estimating mortality in advance must be found. Mortality during a period of years is correlated with the same factors

as rate of growth. Consequently these correlations were made for 5-, 10-, and 15-year periods (table 6). Significant correlations existed in every case. From these, regression equations were computed by means of which the mortality for periods up to 15 years can be estimated (table 7).

TABLE 6.—*Mortality correlations for 5-, 10-, and 15-year periods*

Mortality (square feet of basal area) correlated with—	For 5-year period [1]		For 10-year period [2]		For 15-year period [3]	
	Correlation coefficient	Error	Correlation coefficient	Error	Correlation coefficient	Error
(1) Percent of average density at beginning of period	0.630	±0.056	0.777	±0.046	0.767	±0.066
(2) Square feet of basal area per acre present at beginning of period	.349	±.082	.501	±.088	.518	±.117
(3) Percent of average density at beginning of period and square feet of basal area per acre present at beginning of period	.713	±.046	.837	±.036	.837	±.050

[1] 115 cases.
[2] 73 cases.
[3] 39 cases.

TABLE 7.—*Regression equations for predicting mortality*

Period	Regression equation [1]	Standard error of estimate	Number of cases
5 years	$X_1=0.5878X_2-0.1841X_3-21.0$	5.4	115
10 years	$X_1=1.0166X_2-0.2946X_3-36.8$	6.5	73
15 years	$X_1=1.3310X_2-0.4578X_3-32.0$	8.4	39

[1] X_1=mortality (basal area in square feet per acre of trees which will die during period). X_2=percentage of average density at beginning of period. X_3=square feet of basal area per acre present at beginning of period.

As in the case of the growth correlations, alinement charts were constructed to facilitate the use of these equations. From these the values in table 8 were read. Thus a method is now available whereby the amount to remove in a thinning can be estimated. It must be borne in mind, however, that the removal of just those trees which are expected to die under natural conditions is the minimum requirement necessary if the stand is to be kept as nearly fully stocked as possible. The use of understocking for increasing the growth rate may have considerable merit, as is indicated in table 4. This phase of the problem, however, has not been considered in this study.

TABLE 8.—*Basal-area mortality in 5, 10, and 15 years by basal area per acre present at beginning of period, for stands of different densities* [1]

5 YEARS

Percentage of average density	Mortality by basal area which at beginning of period was—															
	60 square feet	70 square feet	80 square feet	90 square feet	100 square feet	110 square feet	120 square feet	130 square feet	140 square feet	150 square feet	160 square feet	170 square feet	180 square feet	190 square feet	200 square feet	
55	0.2	0.0														
60	3.5	1.5	0.0													
65	6.2	4.3	2.5	0.7	0.0											
70	9.2	7.5	5.6	3.7	1.8	0.0										
75	12.0	10.3	7.5	6.7	4.8	2.9	1.1	0.0								
80	15.0	13.3	11.3	9.5	7.8	6.0	4.0	2.0	0.2	0.0						
85	18.1	16.3	14.4	12.6	10.8	9.0	7.0	5.2	3.4	1.5	0.0					
90	21.0	19.0	17.3	15.5	13.6	11.5	10.0	8.1	6.2	4.4	2.5	0.6	0.0			
95	24.0	22.0	20.2	18.3	16.5	14.6	12.9	11.0	9.1	7.2	5.5	3.6	1.8	0.0		
100	26.9	25.0	23.0	21.2	19.4	17.5	15.8	13.9	11.9	10.1	8.2	6.4	4.7	2.9	1.0	
105	29.8	28.0	26.1	24.2	22.5	20.5	18.8	16.8	15.0	13.1	11.1	9.5	7.8	6.0	4.0	
110	32.7	30.8	29.0	27.2	25.5	23.5	21.5	19.8	17.9	16.0	14.1	12.1	10.5	8.8	6.9	
115	35.5	33.8	31.9	30.0	28.3	26.5	24.7	22.8	20.9	19.0	17.0	15.3	13.5	11.5	9.8	
120	38.5	36.7	34.9	33.0	31.1	29.4	27.5	25.8	23.9	22.0	20.0	18.1	16.4	14.5	12.7	
125	.....	39.5	37.8	35.9	34.0	32.1	30.2	28.6	26.9	25.0	23.0	21.1	19.3	17.5	15.8	
130	.....	.....	.....	38.8	36.9	35.0	33.2	31.4	29.6	27.9	26.0	24.0	22.1	20.2	18.5	
135	.....	.....	.....	.....	39.9	38.0	36.1	34.2	32.5	30.7	29.0	27.0	25.2	23.2	21.4	
140	.....	.....	.....	.....	.....	.....	39.1	37.1	35.5	33.7	31.9	30.0	28.0	26.2	24.4	
145	.....	.....	.....	.....	.....	.....	.....	40.2	38.5	36.7	34.7	32.9	31.0	29.2	27.4	

10 YEARS

60	6.6	3.5	0.8	0.0											
65	11.5	8.6	5.8	3.0	0.0										
70	16.9	13.8	10.8	7.9	5.0	2.0	0.0								
75	21.6	19.0	16.0	13.0	9.9	7.1	4.0	1.2	0.0						
80	26.9	23.8	20.8	18.2	15.0	12.0	9.0	6.1	3.2	0.5	0.0				
85	31.8	28.9	26.0	23.0	20.0	17.2	14.2	11.1	8.2	5.3	2.8	0.0			
90	37.0	33.8	30.9	28.0	25.0	22.1	19.2	16.6	13.4	10.5	7.6	4.8	1.9	0.0	
95	42.0	38.9	36.0	33.0	30.1	27.2	24.3	21.3	18.6	15.7	12.7	9.7	6.8	3.8	1.0
100	47.0	44.1	41.2	38.2	35.2	32.2	29.3	26.6	23.5	20.6	18.0	14.9	11.9	9.0	6.0
105	52.1	49.1	46.1	43.2	40.2	37.3	34.3	31.4	28.4	25.6	22.6	19.8	17.0	14.0	11.0
110	57.2	54.3	51.2	48.3	45.3	42.4	39.5	36.6	33.3	30.5	27.5	24.6	21.8	19.0	16.1
115	.....	59.2	56.5	53.5	50.5	47.6	44.7	41.8	38.8	35.9	32.8	29.8	27.0	23.9	21.0
120	.....	.....	.....	58.7	55.5	52.8	49.7	46.7	43.8	40.9	37.9	34.8	31.9	29.0	26.0
125	.....	.....	.....	.....	.....	57.9	54.7	51.7	48.7	45.9	42.9	40.0	37.0	33.9	31.0
130	.....	.....	.....	.....	.....	.....	59.9	57.0	54.0	51.0	48.0	45.0	42.0	39.0	36.0
135	.....	.....	.....	.....	.....	.....	.....	.....	59.0	56.0	53.0	50.0	47.0	44.0	41.1
140	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	58.1	55.1	52.1	49.1	46.2

15 YEARS

60	30.2	27.2	24.1	21.0	18.0	15.0	12.0	8.9	5.9	2.9	0.0				
65	34.9	31.9	28.7	25.4	22.5	19.4	16.2	13.4	10.4	7.1	4.0	1.0	0.0		
70	39.2	36.2	33.0	30.0	27.0	23.9	20.9	17.8	14.6	11.7	8.5	5.5	2.5	0.0	
75	43.5	40.5	37.5	34.4	31.4	28.3	25.2	22.2	19.0	16.0	12.9	9.9	6.8	3.8	0.8
80	48.2	45.0	41.9	38.9	35.9	32.8	29.8	26.7	23.7	20.6	17.4	14.3	11.2	8.1	5.0
85	52.6	49.4	46.4	43.3	40.1	37.2	34.1	31.0	27.9	24.9	21.9	18.8	15.7	12.7	9.7
90	57.1	54.0	50.8	47.8	44.8	41.7	38.6	35.6	32.5	29.4	26.3	23.2	20.1	16.9	14.0
95	61.5	58.5	55.5	52.4	49.2	46.1	43.0	39.9	37.0	33.9	30.8	27.7	24.6	21.5	18.4
100	65.8	62.8	59.7	56.9	53.8	50.4	47.4	44.3	41.2	38.2	35.0	32.0	28.9	25.9	22.9
105	70.1	67.5	64.1	61.0	58.0	55.0	51.9	48.9	45.8	42.7	39.5	36.5	33.4	30.3	27.2
110	74.5	71.5	68.5	65.5	62.4	59.3	56.5	53.3	50.1	47.0	43.9	40.9	37.9	34.8	31.8
115	78.9	75.8	72.8	69.8	66.9	63.8	60.8	57.8	54.8	51.7	48.5	45.3	42.2	39.1	36.0
120	83.5	80.4	77.2	74.1	71.1	68.2	65.2	62.0	59.0	56.0	52.9	49.8	46.8	43.7	40.6
125	88.0	84.9	81.7	78.6	75.2	72.5	69.5	66.5	63.4	60.2	57.4	54.3	51.1	48.0	45.0
130	92.1	89.2	86.2	83.0	80.0	76.9	73.9	70.9	68.0	64.9	61.7	58.7	55.7	52.5	49.4
135	96.8	93.7	90.5	87.6	84.5	81.3	78.2	75.1	72.1	69.1	66.1	63.0	60.0	57.0	54.0
140	.....	98.0	94.9	91.9	89.0	86.0	82.9	79.8	76.6	73.6	70.6	67.8	64.5	61.4	58.4

[1] Block indicates extent of basic data.

There still remained, however, the problem of how far ahead it is wise to estimate mortality in order to determine how much to remove in any one thinning; in other words, what should be the maximum interval between thinnings? Common sense indicates that there must be a limit to the amount that can be removed at any one time and yet leave the remaining stand in good condition. (Quality increment is not considered here, although it is decidedly a factor to be reckoned with in actual thinning operations.)

Tests were made to see how far ahead the rate of growth for any one period was correlated with the present density and basal area. The rate of growth for successive 5-year periods was correlated with density and basal area present at the beginning of the first period. The results are presented in table 9. The first and second 5-year periods show very significant correlations, the third period is just barely significant, while the fourth period shows no significance. The general level of significance of the correlation coefficients was lowered in each succeeding 5-year period, indicating that 15 years is the maximum period during which rate of growth is correlated with present density and basal area. An interval of 15 years should be the maximum between thinnings, and the shorter the interval the better the result.

TABLE 9.—*Correlations between growth for succeeding 5-year periods and density and basal area at beginning of first period*

5-year period	Correlation coefficient	Computed z [1]	Tabular value of z at 1-percent level	Tabular value of z at 5-percent level	Significance	Cases
						Number
First	0.5025	1.4701	0.8025	----------	Significant	115
Second	.5203	1.2749	.8025	----------	do	73
Third	.4000	.6112	----------	0.5994	Barely significant	39
Fourth	.3536	.037	----------	.6594	Not significant	16

[1] Fisher's z test used to show significance of correlation coefficients (*4*).

Attention should be called to the fact that the errors of estimate of these predicting equations are large when thought of in terms of growth. The error of estimate of 7.4 when compared with the values in that part of table 4 for the 5-year period indicates a standard error of from one-third to two or even three times the growth. The errors of estimate of mortality are also high, though not quite as high as the growth errors. However, when one remembers that the stands for which the predictions might be made contain 80 to 200 square feet of basal area per acre, the prediction of the amount that will be present at the end of any period has an error which is only a small percentage of the total stand.

Though the errors of estimate are high, the significant trends of growth and mortality with basal area and density, as shown in tables 4 and 8, are important. Because the equations are computed from natural stands in which mortality and the resultant correlated growth (table 5) are subject to considerable variation, is indicated by the high errors of estimate, it will be possible to attain much closer correlation when dealing with managed stands. In other words, by removing mortality in advance, the effect of this factor upon rate of

growth is controlled. In addition, more perfect spacing is gained by the thinning operation. Thus, by the time a second thinning is necessary much more accurate estimates of future growth will be possible.

SUMMARY

Mortality and growth rate in even-aged stands of loblolly pine are shown to be significantly correlated with stand density and basal area. If the present stand density and basal area are known, the future rate of growth and mortality may be predicted from tables for 5-, 10-, and 15-year periods. A table is also presented for estimating stand density from number of trees per acre and their average diameter. Although the errors of estimate are high in predicting growth and mortality in natural stands from these tables, the authors believe that much more accurate results are possible in managed stands. Correlations of growth for successive 5-year periods with density and basal area present at the beginning of the first period indicate that 15 years should be the maximum interval between thinnings, and that the shorter the interval the better the results.

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THE TOXICITY OF CERTAIN INSECTICIDES TO THE CHINCH BUG [1]

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INTRODUCTION

Since its discovery in 1783, the chinch bug *Blissus leucopterus* (Say), has been recurrently abundant over wide areas of North America and efforts upon an extensive scale have frequently been made to control it. Not until a century after its discovery, however, was the use of chemicals for its suppression seriously considered; and the literature since 1883 reveals many attempts to control the chinch bug by means of sprays and dusts. At the present time, mechanical barriers, cultural practices, and the planting of resistant crops are chiefly relied upon to hold the insect in check, but chemicals are still of important though minor consideration.

The purpose of this investigation was twofold: (1) To examine for insecticidal efficiency in the laboratory some of the compounds and mixtures which have been recommended for chinch bug control; and (2) to determine the relative efficiency of some of the newer insecticides in both the laboratory and the field. In addition, it seemed desirable to ascertain, so far as time would permit, the difference in susceptibility of the various nymphal instars and of the adult to each insecticide.

The laboratory experiments were made at Ames, Iowa, from June 7 to July 14, 1934, and the field experiments were made near Osceola, Clarke County, Iowa, during the same period.

HISTORICAL REVIEW

The first attempt of consequence to control the chinch bug by means of sprays was made in 1882 when Forbes (*1*, *13*)[2] experimented with emulsions of kerosene and soap and kerosene and milk at 2.5- to 6.7-percent oil concentrations. The results were favorable; the injury to the corn was reported as negligible. About this time the Riley-Hubbard formula for kerosene emulsion became available (*25*), and was most frequently used thereafter in the preparation of this insecticide. By about 1889 (*14*, *29*), it was quite generally reported that a kerosene emulsion must contain not less than 4 or 5 percent of oil for effective results on the chinch bug. Gossard (*19*) found that emulsions containing from about 7 to 8 percent of kerosene were effective against the younger chinch bugs, but that they would not kill all the winged adults. In 1913 Headlee and McColloch (*22*) reported kerosene emulsion so difficult to prepare and so injurious to corn that it could not be used in practice. Subsequent trials have generally shown that this emulsion cannot be relied upon to control the chinch

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[2] Reference is made by number (italic) to Literature Cited, p. 76.

bug at kerosene concentrations which are uniformly safe for the corn plant.

Other kerosene preparations have been tested against the chinch bug. Forbes (*13*) reported favorably a mixture of kerosene and water containing 3 percent of kerosene which did not injure the plant if it was constantly stirred during application. Stedman (*38*) also employed a mixture of kerosene and water (10 percent of kerosene), applied by means of a special pump, which apportioned and mixed the ingredients. No recent studies of such mixtures have been reported. Forbes (*16*) found a gasoline-soap emulsion of no value against the chinch bug.

Lubricating oils, in emulsions, which have usually contained other toxic substances such as nicotine, have been used on a number of occasions (*7, 23*). Their value is not clear from the results.

Other organic liquids, notably carbon disulphide, coal tar, and turpentine, emulsified with soap, have been used to control chinch bugs. Carbon disulphide was tried by Osborn (*29*), and in the form of an emulsion by Felt and Bromley (*7*). The toxic efficiency was low in these tests. The coal-tar emulsion, diluted with 9 parts of water, was reported (*14*) as effective as kerosene emulsion and it did not injure the corn plant. A turpentine emulsion (*14*) was less effective than the kerosene emulsion.

A solution of soap and water was employed in one of the early attempts to control chinch bugs by means of liquids (*24*). In 1908 Forbes (*15*) found that one-half pound of whale-oil soap in 1 gallon of water destroyed most of the bugs but caused some injury to corn at the "curl" (the growing point of the plant). Three-fourths of a pound of the soap per gallon was still more effective on the bugs and more dangerous to the plant. One and one-half pounds per gallon killed most of the plants to which it was applied, and a concentration of 2 pounds per gallon was fatal to all of the treated corn plants. Headlee and McColloch (*22*) recommended a lower concentration of whale-oil soap (0.16 pound per gallon) which they found did not injure the corn. In 1916 Forbes (*16*) reported the results of experiments with 13 commercial soaps. There was a wide variation in the effectiveness of the samples. A field test demonstrated that a soap concentration of 0.19 pound per gallon will kill about 75 percent of the bugs in corn without injuring the plants. Walton (*44*) in 1917 recommended 0.17 pound of soap powder in 1 gallon of water. Since that time the recommended concentrations of soaps, either laundry or whale-oil, have ranged from about 0.16 to 0.22 pound per gallon (*12, 33, 39*).

Nicotine was first employed against the chinch bug in aqueous decoctions of tobacco (*14, 16*) with no outstanding success. In 1910 and 1911 Forbes (*16*) showed that nicotine sulphate (40 percent of nicotine) in a soap solution was more toxic to chinch bugs and more easily prepared than kerosene emulsion; furthermore, the solution did not injure the corn plant. The effectiveness of the nicotine was greatly increased by the addition of soap. Headlee and McColloch (*22*) in Kansas and Hudson (*26*) in Canada recommended nicotine-sulphate-soap solutions. The more recent practice is to use concentrations of 0.16 (*33*) to 0.5 ounce of nicotine sulphate (40 percent of nicotine) per gallon (*11*) with about 0.64 to 1.28 ounces of soap per gallon. Of other alkaloidal substances, a strong decoction of lobelia was ineffective (*14*).

Nicotine dusts containing 2 or 3 percent of nicotine have been recommended by Flint and Balduf (*11*), Flint and Larrimer (*12*), and others.

Pyrethrum powder was used against the chinch bug by Osborn (*29*), Forbes (*14*), and Bunsen (*2*) without marked success. Gillette (*17*) in 1891 reported tests on chinch bugs and some other insects with a kerosene extract of pyrethrum emulsified with soap, and observed a marked increase in toxicity from the effects of the substance extracted from the pyrethrum. These experiments foreshadowed the extensive use of kerosene extracts of pyrethrum for insect control in more recent times. In 1931 Felt and Bromley (*7*) reported tests with several proprietary insecticides containing pyrethrum which they found only moderately effective against chinch bugs at the concentrations used.

On a number of occasions cyanide compounds have been shown to kill chinch bugs when applied directly to infested plants. Flint (*10*) killed large numbers of these insects with solutions of sodium cyanide, but concluded that the material was too poisonous to be used in practice. Flint and Balduf (*11*) employed commercial calcium cyanide as a dust. It was very poisonous to the bugs, but it injured the corn and was too costly for general use. Similar results with calcium cyanide were reported by Haseman and Bromley (*21*), Swenk (*39*), and Felt and Bromley (*7*).

Various arsenicals, such as arsenious oxide, london purple, paris green (*14*), and sodium arsenite (*10*), have also been used. Most of these have shown low insecticidal value, although some mortality has been observed from both arsenious oxide (*14*) and sodium arsenite (*10*). A certain degree of effectiveness for lead acetate and mercuric chloride was claimed by Forbes (*14*) and Flint (*10*). Ferrous sulphate, applied as a spray, was worthless (*16*), as were also limewater and gas lime (*14*).

LABORATORY EXPERIMENTS

BIOLOGICAL MATERIAL

Chinch bugs, gathered in large numbers from growing corn (*Zea mays* L.), were placed in a cage with glass and screen sides where they were retained until used. The bugs were supplied at all times with young, growing corn plants. New lots of chinch bugs were collected at least once a week, sometimes oftener. Groups of the bugs, representing all of the stages present in the population at any one time and numbering a thousand or more individuals, were transferred daily from the stock cage to large glass dishes. The escape of the insects was prevented by a film of vaseline placed upon the inside of the dishes just below the rim. Samples of bugs for experimental use were removed at random from these dishes.

CHEMICAL COMPOUNDS AND MIXTURES

Thirteen chemical compounds or mixtures were used in the laboratory experiments, five of which were soaps, two kerosene preparations, one nicotine, one piperidine, one a pyrethrum extract, and the remainder rotenone solutions and extracts of derris.

The following soaps were employed: Sodium oleate (United States Pharmacopoeia neutral powder); sodium-base laundry soap (25.5 percent moisture); potassium oleate (a laboratory product prepared from oleic acid U. S. P. and chemically pure potassium hydroxide); commercial potassium oleate (containing 65.4 percent of solids, of which 33.2 percent was potassium oleate); commercial potassium fish-oil soap (34.6 percent of soap).

The kerosene preparations consisted of a kerosene emulsion of the following composition: Kerosene (gravity, American Petroleum Institute 41.4°, boiling range 378°–516° F.) 80 g, potassium fish-oil soap (see above) 10 g, water 10 g; and a kerosene-naphthalene emulsion that contained kerosene (as above) 72 g, naphthalene 8 g, potassium fish-oil soap (as above) 10 g, and water 10 g. The emulsions were made by a cold-stir method (*30*) with mortar and pestle. In the emulsion which contained naphthalene, the naphthalene was dissolved in the oil by the use of gentle heat before emulsification.

The nicotine and piperidine were both quite pure products. The former was redistilled in vacuo from 95-percent nicotine; the latter was a commercial product, having a boiling range of 105.5° to 107.5° C.

The pyrethrum extract was made from freshly ground pyrethrum flowers which were extracted for 18 hours in a Soxhlet apparatus with low-boiling petroleum ether, the ether afterwards being removed in vacuo. Then the residue was taken up in acetone. Quantitative determination by the method of Seil (*32*) gave total pyrethrins 0.82 percent, of which there was 0.34 percent of pyrethrin I and 0.48 percent of pyrethrin II.

The rotenone was a purified product, the melting point of which was 163° C.

Two derris extracts were made from a commercial derris powder which upon analysis, when air-dried, gave the following average percentage figures: Moisture, 5.48; rotenone, 5.70; total methoxyl groups (CH_3O), 9.16. The first extract, with acetone, was made in a Soxhlet apparatus, extraction being continuous for 6 hours. The extract contained 4.47 percent of CH_3O and the residue 5.13 percent, the total CH_3O being 9.60 percent. The second extract, with carbon tetrachloride, was also made in a Soxhlet apparatus; but the extraction time was 18 hours. This extract contained 3.22 percent of CH_3O and the residue 6.14 percent, the total CH_3O being 9.36 percent, which is in good agreement with that of the powder and of the combined acetone extract and residue. Other extracts, with acetone and carbon tetrachloride, were made in the same manner from this derris powder.

EXPERIMENTAL PROCEDURE

The method used in applying the toxic solutions and liquid mixtures in the form of a fine spray has been described (*4*). The soap-nicotine and piperidine solutions were made up to contain known percentage concentrations (grams per 100 cc of the solution) of the respective compounds. Solutions of these two compounds contained 0.25 percent of sodium oleate which served as a spreading agent. The kerosene emulsions were diluted to a known percentage of kerosene (grams per 100 g of the emulsion). The pyrethrum and derris extracts

were diluted to contain the desired percentage concentrations (grams per 100 cc) of total pyrethrins and rotenone respectively in a solution containing 75 percent by volume of acetone. The rotenone was dissolved in pure acetone to form stock solutions from which the solutions for the tests in 75 percent by volume of acetone were made up as desired. The stock solutions of rotenone were freshly prepared every 3 days.

The solutions or emulsions were delivered at a nearly uniform rate and a constant pressure of 6 pounds per square inch as a spray into the container which held the sample of insects. The time of delivery was 30 seconds. Under these conditions, the nozzle delivers into the container 1.80 g (average) of distilled water or 1.84 g (average) of kerosene emulsion containing 20 percent of oil. Because of the rather high volatility of the 75 percent acetone solution, the amount of this liquid delivered from the nozzle in 30 seconds was determined rather than the amount which fell in the container. This amount was 2.72 g (average). The container which held the insects of course received a smaller quantity.

The samples of insects used in the tests were drawn into a glass tube by gentle suction and then carefully expelled into the container. The sampling was done at random from a population which usually numbered many thousands.

After treatment, the insects were removed to glass tumblers with a camel's-hair brush moistened with the spray liquid in order to disturb the spray film on the insects as little as possible. Each tumbler was supplied with a piece of fresh young corn foliage. As the insects were removed from the container, they were segregated, according to the stage of development, into four groups: a first- and second-instar group; a third- and fourth-instar group; a fifth-instar group; and an adult group. Each tumbler was placed in the center of a pan of water to prevent the escape of the insects. A sample representing a single instar or adult group usually contained 15 to 75 insects, and with few exceptions the tests were replicated five or six times.

Controls, which varied according to the solvent used, were employed in all experiments. In tests of the compounds which were dissolved in water or in 0.25-percent sodium oleate solution, control groups were sprayed only with these solvents. Other groups sprayed with solutions containing 75 percent by volume of acetone served as controls for their respective tests. In addition, many tests were made with groups of insects which received the same amount and kind of handling as the experimental groups except that they were given no insecticide treatment. In this manner a check was maintained on the viability of the insect populations as they were brought in from the field.

Mortality in the control groups was generally low. For those sprayed with water or those without treatment, the mean mortality ranged from 3.2 percent (adult) to 16.3 percent (first- and second-instar group). Groups which were sprayed with the 75-percent acetone solution varied in mean percentage of mortality from 2.0 (adult) to 13.7 (third- and fourth-instar group); the first- and second-instar group was the most susceptible, the mean mortality being 38.2 percent.

The value for 0.25-percent sodium oleate solution may be read from the curve shown in figure 1.

All experimental percentages of mortality were calculated to the net mortality (N) by the formula, $N=\frac{(x-y)}{x}100$, where x is the percentage of insects alive in the control group and y the percentage alive in the experimental group.

RESULTS

Soap Solutions

Information concerning the toxicity to the early instars (first- and second- and third- and fourth-instar groups) is available only for sodium oleate, potassium fish-oil soap, and commercial potassium oleate. The data for the first two instar groups treated with these

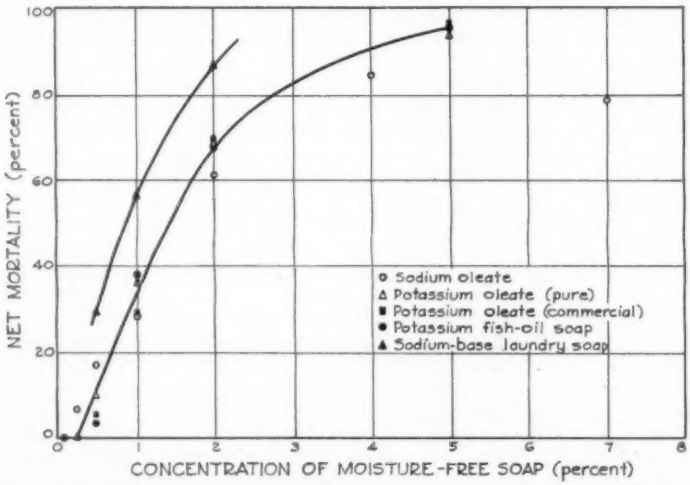

FIGURE 1.—Net mortality of adult chinch bugs when various concentrations of five different soaps were applied. These and subsequent curves were fitted by eye to the mortality data.

three soaps at the three concentrations (0.5, 2.0, 5.0 percent) have been analyzed by the method of analysis of variance (*37*). The results are brought together in table 1.

The next to the last line of table 1 contains that part of the variation which is not assigned to soaps, concentrations, or instars. It is termed the experimental error. To obtain the significance or nonsignificance of any other source of variation treated in the analysis, the ratio of the mean square of that source to the mean square of the experimental error is determined. From this ratio the degree of significance of the specified source of variation is obtained by reference to Snedecor's table (*37*). Table 1 shows that only the variation between concentrations of the soaps is significantly different, the chances being less than 1 in 100 that the difference is assignable to errors of random sampling. This difference was, of course, expected. The variation

between means of soaps is indicated by $F=5.184$, which corresponds to a probability greater than 5 in 100; therefore the difference in mean mortality of the soaps under the specified conditions is too small to be considered significant.

TABLE 1.—*Analysis of variance of percentage mortality for chinch bugs of the first 2 instar groups,*[1] *and for the fifth instar and adults, when 3 and when 5 soaps, respectively, were used at 3 concentrations*

FIRST 2 INSTAR GROUPS; 3 SOAPS

Source of variation	Degrees of freedom	Sum of squares	Mean square	Interpretation [2]
Between means of soaps	2	1,487.98	743.99	Not significant.
Between means of concentrations	2	14,091.26	7,045.63	Highly significant.
Between means of instars	1	20.06	20.06	Not significant.
Interactions:				
Soap-concentration	4	227.38	56.84	Do.
Soap-instar	2	434.24	217.12	Do.
Concentration-instar	2	8.71	4.36	Do.
Second-order interaction-remainder (experimental error).	4	574.04	143.51	
Total	17	16,843.67		

FIFTH INSTAR AND ADULT; 5 SOAPS

Between means of soaps	4	1,036.59	259.15	Significant.
Between means of concentrations	2	22,360.09	11,180.04	Highly significant.
Between means of instars	1	622.54	622.54	Significant.
Interactions:				
Soap-concentration	8	519.41	64.93	Not significant.
Soap-instar	4	293.37	73.34	Do.
Concentration-instar	2	508.50	254.25	Nearly significant.
Second-order interaction-remainder (experimental error).	8	500.31	62.54	
Total	29	25,840.81		

[1] First and second and third and fourth groups.
[2] Significant, not more than 5 chances in 100; highly significant, not more than 1 chance in 100.

A similar analysis has been made of the mortality data for the five soaps (sodium oleate, laundry soap, potassium oleate, commercial potassium oleate, potassium fish-oil soap) on the fifth-instar nymphs and the adults at three concentrations (0.5, 1.0, 2.0 percent). The results are shown in table 1.

Again the high significance of the variation between means of concentrations is of no interest because expected, but the significance of the variation between means of soaps and means of instars is important. It shows (1) that there is a difference in mortality attributable to the kind of soap used and (2) that there is a difference in mortality depending on the stage of the insect employed. A further analysis of the mortality data shows that the sodium-base laundry soap is significantly more toxic than the other soaps (fig. 1), and that between the remaining four soaps no significant differences exist. A separate analysis of the concentration and instar data reveals at 0.5-percent soap concentration no significant difference in resistance between the fifth-instar nymphs and the adults, whereas at 1.0- and 2.0-percent concentrations the adults are definitely the more resistant.

Kerosene Emulsions

Only partial curves of mortality were determined for the kerosene emulsion and kerosene-naphthalene emulsion. These are shown in figure 2. Even at the highest oil concentration used (20 percent by weight), kerosene emulsion produced a mortality of the adult bugs below 50 percent; younger stages were less resistant, but the mortality for them was below 80 percent. The addition of naphthalene to the oil in the ratio of 10 percent of the oil-naphthalene component materially increased toxicity, but even at 20-percent concentration (18 percent of oil, 2 percent of naphthalene) only slightly more than 80 percent of the adults and 95 percent or less of the younger stages were killed.

Nicotine

Nicotine base in a solution containing 0.25 percent of sodium oleate was noticeably toxic to chinch bugs. Remarkable, however, was the comparatively greater resistance of the younger stages to this alkaloid. Although the other insecticides used in these tests were more toxic to the younger stages, nicotine was unique in being significantly more toxic to the adult. Toxicity data for nicotine (fig. 3) are not available through the midportion of the curve, but the concentration for 50-percent mortality may be roughly estimated as follows: Adult, 0.08 percent; fifth instar, 0.45; third- and fourth-instar group, 0.25. On this basis, the adult is about five and one-half times as susceptible to nicotine as the fifth instar and about three times as susceptible as the third- and fourth-instar group. The first- and second-instar group appears to occupy an intermediate position between the fifth- and the third- and fourth-instar groups with respect to its susceptibility to nicotine.

Most of the previous tests of nicotine against chinch bugs have been made in soap solutions of about 0.5-percent concentration or above. Such concentrations of soap are in themselves appreciably toxic to these insects; furthermore, as shown above, soap is more toxic to the younger stages than to the adult. Therefore, the addition of soap to the mixture will largely correct the low toxicity of nicotine for the younger bugs.

Piperidine, Pyrethrum Extract, and Rotenone

Piperidine, an organic base resembling nicotine in certain respects, was also tested in 0.25-percent sodium oleate solution. Only four concentrations of piperidine were used on the third- and fourth-instar group and the fifth instar. Even at a concentration of 5 percent the mortality was little greater than that of the 0.25-percent soap solution. There were indications that the older bugs were more susceptible than the younger to this compound but the differences were not marked and the data are too meager to justify comparisons at these low levels of toxicity.

Pyrethrum extract was applied in the 75-percent acetone solution to adult chinch bugs only, the percentage concentrations being 0.0005, 0.0025, 0.025, 0.05, and 0.25. In figure 4, the curve drawn freehand passes through the point of 50-percent mortality at a concentration of about 0.0025 percent of total pyrethrins. The acetone solution itself has a negligible toxicity for the adult chinch bug (p. 64).

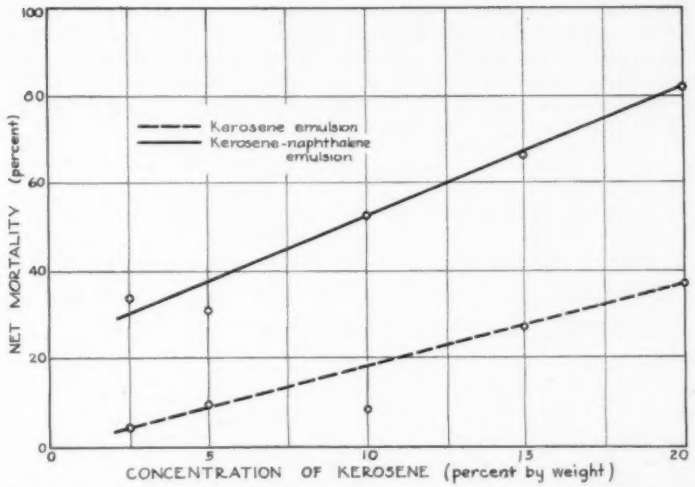

FIGURE 2.—Net mortality of adult chinch bugs when various concentrations of kerosene emulsion and of kerosene-naphthalene emulsion were applied.

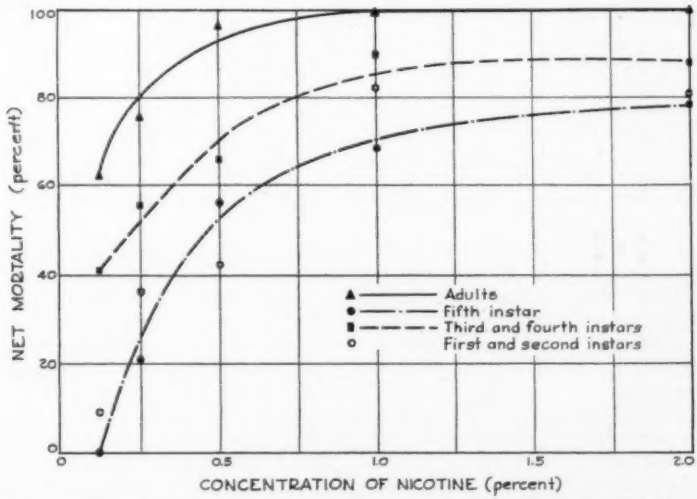

FIGURE 3.—Net mortality of adult chinch bugs and of nymphs of the fifth-, third-, and fourth-, and first- and second-instar groups, when various concentrations of nicotine, with 0.25 percent of sodium oleate as spreader, were applied.

Rotenone, also dissolved in the 75-percent acetone solution was applied in five concentrations (0.005 to 1.0 percent) to chinch bugs of the first- and second-, third- and fourth, and fifth-instar groups. Adults were not included because preliminary trials with them had shown very low mortality. The curves (fig. 5) indicate high mortality only to the youngest bugs at 0.5 g per 100 cc. The fifth-instar nymphs appear to be very resistant; the mortality did not exceed 5 percent over a wide range of concentrations.

Extracts of Derris

Acetone and carbon tetrachloride extracts of pulverized derris root were tested for toxicity on chinch bugs of various ages. The data for both extracts are complete only for the adults; in addition, the ace-

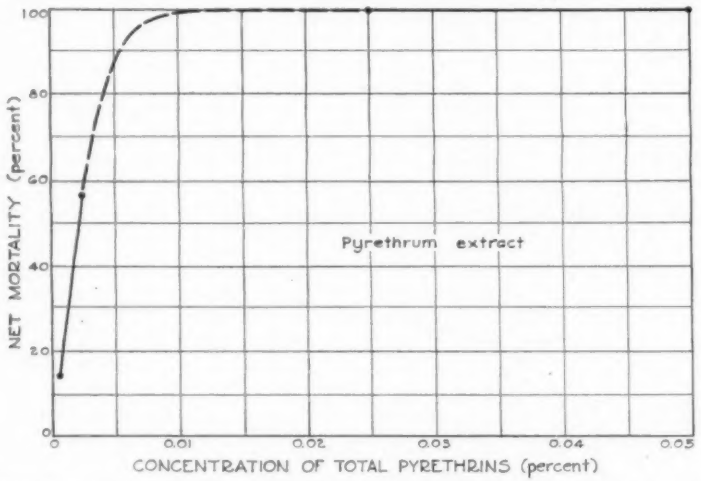

Figure 4.—Net mortality of adult chinch bugs when various concentrations of pyrethrum extract in 75 percent by volume of acetone were applied.

tone extracts were tested on the third- and fourth-instar group and the fifth-instar group. Extracts made with these two solvents were investigated because it had been shown (*27*) that acetone removes a greater proportion of solid material from the derris root than does carbon tetrachloride. It was believed that this quantitative difference in the dissolved solids of the two extracts might be expressed by a difference in toxicity.

Both extracts were held in acetone solution until ready for use, when they were diluted with water to 75 percent by volume of acetone. The solutions were made up to contain 0.001, 0.005, 0.05, 0.1, and 0.5 percent of rotenone, calculated from the percentage of rotenone in the derris powder (p. 62).

The results are shown in figure 6. There are probably no significant differences between the net mean percentages of mortality for equivalent instar groups and rotenone concentrations. The net mean mortalities for the 0.005-percent concentrations differ by 13.3 units, and

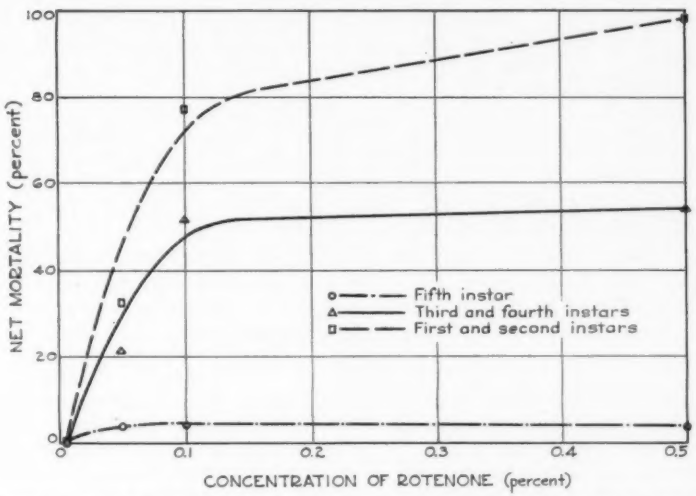

FIGURE 5.—Net mortality of chinch bug nymphs of the fifth, third, and fourth, and first and second instars when various concentrations of rotenone, in 75 percent by volume of acetone were applied.

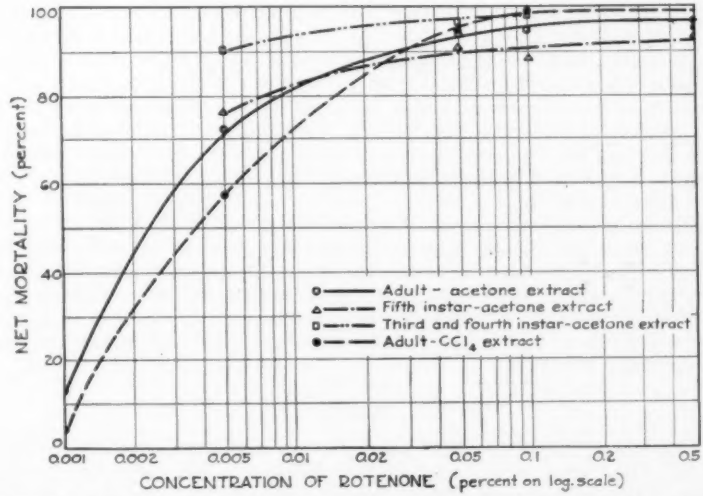

FIGURE 6.—Net mortality of adult chinch bugs and of nymphs of the fifth and of the third and fourth instars, when various concentrations (on a basis of rotenone) of derris extract in acetone or in carbon tetrachloride were applied.

comparison of these means by the small sample method of Fisher (*8*) shows this difference to be significant ($P=0.05$). However, the χ^2 test for goodness of fit reveals that the mortality figure, 72.4 percent for the acetone extract, is open to suspicion. For this reason, no claim of a difference in toxicity for the two derris extracts seems justifiable from the present toxicity data.

On the basis of rotenone content, derris extracts were about as toxic as pyrethrins and much more toxic than rotenone. There were no significant differences in toxicity attributable to differences in the concentration of methoxyl groups in the extracts, although the carbon tetrachloride extract contained only about 72 percent of the methoxyl content of the acetone extract. Campbell, Sullivan, and Jones (*3*), on the other hand, have observed a rather close agreement of toxicity with methoxyl content in kerosene extracts of derris, when the house fly (*Musca domestica* L.) was the test insect.[3]

FIELD EXPERIMENTS

EXPERIMENTAL PROCEDURE

Sprays and dusts were used in field experiments. The sprays were applied with a hand sprayer capable of developing a pressure of about 90 pounds per square inch. In applying the liquids, an attempt was made to cover the plants with a complete and even film. The dusts were applied with a hand duster. The quantities dusted on each plot were determined and calculated in pounds per acre for a standard planting of corn (3,556 hills per acre in rows 3.5 feet apart in the rows and between the rows).

Each plot which received a treatment consisted of a quadrat of four adjacent hills of corn surrounded by a barrier of creosote to prevent migration of the bugs into or away from the enclosed area. The plots were separated from each other by several rows of untreated corn to eliminate the effect of drifting of the insecticides. A control plot of equal size was chosen adjacent to each group of experimental plots. No particular plot arrangement was used and no statistical treatment has been given the experimental data. It will be noted that the populations varied in the proportions of the different stages present on the different dates.

The percentage of mortality was estimated within 2, 4, and 24 hours after treatment by sampling at random the population under experiment. The mortality from other causes in the controls was so low that it was not taken into consideration in estimating the experimental mortality. The mortality figures, though not so precise as those derived from the laboratory data, are considered adequate for this preliminary study.

RESULTS

Sprays

The three soaps used in the field experiments (table 2) showed, under the conditions, very little difference in toxicity to the bugs. The apparently greater toxicity of the laundry soap in the

[3] Papers by Jones, Campbell, and Sullivan (*28*) and by Tattersfield and Martin (*45*) have appeared since this was written. Jones et al. find neither the rotenone nor the methoxyl content of the extract from a rotenone-bearing plant a satisfactory measure of insecticidal efficiency against the house fly. The best agreement of the analytical figures with toxicity was obtained by taking the difference between the methoxyl values and the approximate values for toxicarol. Tattersfield and Martin found a somewhat closer agreement of toxicity with the dehydro compounds or the rotenone plus the dehydro compounds in the samples of derris examined. The test insect was the bean aphid (*Aphis rumicis* L.).

laboratory was not borne out in the less rigid field tests. In its effect on the plant, however, the laundry soap appeared to be superior to the others, a 1-percent solution producing less injury to the corn than the corresponding concentration of the other soaps. The results suggest that soaps of the higher saturated fatty acids (palmitic and stearic acids) may be less injurious to the corn plant and equally as toxic or even more toxic to the chinch bug than soaps made from oleic acid. The alkalinity of the soaps may also be a factor in producing the injury.

Some of the field results with lubricating-oil emulsions (table 2), especially those which contained naphthalene, are interesting. Because of the known delayed effect of petroleum oils of high viscosity on many plants, it is not advisable to stress the importance of these results.

The field results with nicotine (table 2) are in substantial agreement with those obtained in the laboratory. About 0.2 percent of nicotine in 0.3-percent soap solution was necessary for a mortality in 24 hours of more than 90 percent of the younger bugs. The frequently recommended proportions of 1 pint of nicotine sulphate (40 percent of nicotine) and 3 to 4 pounds of soap to 50 gallons of water are equivalent approximately to 0.1 percent of nicotine in a 0.5- to 1.0-percent range of soap solution (moisture-free basis). By keeping the soap concentration within these limits, injury to corn is minimized, and at the same time a fair degree of soap toxicity is maintained. The results with dipyridyl oil paste were of particular interest because of the fact that efficient concentrations with soap did not injure the plants. The results are, of course, preliminary.

In the field experiments, pyrethrum extract was used in the proportions of 0.0021 and 0.0042 percent of total pyrethrins in 0.3-percent soap solution. This was equivalent to about 2 and 4 pounds, respectively, of a good pyrethrum powder (0.8 to 1.0 percent of total pyrethrins) in 100 gallons of the soap solution. At the higher concentration, the mortality was satisfactory. Probably better results could be obtained by using a neutral soap or some other nonalkaline spreading agent.

Rotenone, in commercial potassium oleate solution, was efficient in the field only at a concentration of 0.01 percent. The results do not support those obtained in the laboratory with acetone solutions of rotenone, much higher concentrations being required in the soap solutions under field conditions. The experiments with derris extract and with a suspension of derris powder gave rather variable and contradictory results. In some cases the spreaders seemed to exert a large influence upon mortality. In these preliminary field experiments both rotenone and the derris extracts gave rather unfavorable results.

TABLE 2.—*Mortality from insecticides used as sprays in field tests on chinch bugs*

Insecticide	Concentration	Spreader	Concentration	Date	Stage of insects	Estimated mortality, in—			Injury to corn plants
						2 hours	4 hours	24 hours	
	Percent		*Percent*			*Percent*	*Percent*	*Percent*	
Laundry soap (sodium base)	1.0			July 2	Fifth instar	80	80	80	Slight.
Do	2.0			do	do	90	90	90	Moderate.
Do	2.0			June 27	Adult and fifth instar	90	90	95	Slight.
Do	3.0			June 22	Fourth and fifth instars	90	95	95	Severe.
Potassium oleate (commercial)	.3			do	do	25	25	20	None.
Do	.5			July 2	Fifth instar	50	50	50	Moderate.
Do	1.0			do	do	85	85	85	Severe.
Do	1.0			June 22	Fourth and fifth instars	80	80	85	Do.
Do	2.0			July 2	Fifth instar	90	90	90	Do.
Potassium fish-oil soap	.5			do	do	65	65	65	Slight.
Do	1.0			do	do	90	85	85	Moderate.
Do	2.0			do	do	95	95	95	Severe.
Kerosene emulsion [1]	4.0			June 21	Fourth and fifth instars	85	80	[2] 60	Do.
Do	8.0			do	do	95	80	60	Do.
Lubricating-oil emulsion, 85 viscosity	2.0			June 8	Second and third instars	15	15	60	Slight.
Lubricating-oil emulsion, 120 viscosity	2.0			June 21	Fourth and fifth instars	75	75	70	None.
Lubricating-oil emulsion, 120 viscosity plus naphthalene (8 percent)	2.0			do	do	90	90	85	Do.
Fuel-oil emulsion	20.0			June 27	Adult and fifth instar	98	98	98	Severe.
Pine-oil emulsion [1]	2.0			June 15	Third and fourth instars	75	75	75	Do.
Nicotine sulphate; 0.05 as nicotine		Commercial potassium oleate	0.3	June 8	Second and third instars	40	30	30	None.
0.10 as nicotine		do	.3	do	do	80	75	75	Do.
Do		do	.3	June 21	Fourth and fifth instars	70	75	90	Do.
0.2 as nicotine		do	.3	do	Second and third instars	95	95	95	Do.
Do		do	.3	do	Fourth and fifth instars	95	95	95	Do.
0.4 as nicotine		Laundry soap	1.0	June 27	Adult and fifth instar	98	98	98	Very slight.
Dipyridyl oil paste [3]	.5	Commercial potassium oleate	.3	June 22	Fourth and fifth instars	70	75	80	None.
Do	.78	do	.3	June 15	Third and fourth instars	80	85	95	Do.
Do	1.0	do	.3	June 22	Fourth and fifth instars	80	80	90	Do.
Pyrethrum extract:									
0.0021 as total pyrethrins		do	.3	July 7	Adult and fifth instar	80	80	85	Do.
0.0042 as total pyrethrins		do	.3	do	do	85	85	90	Do.
Rotenone in acetone	.005	do	.3	June 15	Third and fourth instars	60	60	50	Do.
Do	.005	do	.15	June 8	Second and third instars	20	30	50	Do.
Do	.01	do	.3	June 21	Fourth and fifth instars	90	90	90	Do.
Do	.01	do	.3	June 8	Second and third instars	90	90	90	Do.

Acetone extract of:									
Derris root [4]	.004	Molasses	2.5	July 6	Adult and fifth instar	75	80	90	Do.
Do	.008	do	2.5	do	do	75	70	70	Very slight.
Do	.028	do	2.5	July 7	do	5	20	50	None.
Do	.028	Glue	1.0	do	do	5	20	50	Do.
Do	.002	Commercial potassium oleate	.3	June 15	Third and fourth instars	5	10	50	Do.
Do	.003	do	.3	July 21	Fourth and fifth instars	75	75	75	Do.
Do	.029	do	.3	July 7	Adult and fifth instar	25	50	80	Do.
Do	.007	Laundry soap	1.0	June 27	do	10	10	20	Do.
Do	.014	None		do	do	10	10	25	Do.
Derris root powder [5]	.5	Glue	.1	July 7	do	5	10	50	Do.

[1] Petroleum oil and pine oil concentrations represent percentage of oil by volume.
[2] In this and in some other experiments there is an apparent decrease of mortality with time ascribable in part to recovery and in part to sampling error.
[3] Dipyridyl oil paste was a commercial product. Concentrations represent grams of paste dispersed in 100 cc of solution.
[4] Concentrations refer to grams of rotenone per 100 cc of spray liquid.
[5] Weight in grams of derris powder per 100 cc.

Dusts

Calcium cyanide dust (table 3) was an efficient means of control only when the amounts used were so large that they caused severe damage to the corn. The other materials employed were relatively valueless. Nicotine dusts have proved efficient, but the carrier must be a substance like calcium hydroxide which does not react with the nicotine to form a stable complex. Bentonite is known to react with nicotine by base exchange (*36*), the nicotine being released with difficulty from the bentonite-nicotine complex. A similar complex is formed when anabasine reacts with bentonite. The experiments with these bentonite dusts are of interest only in showing the inertness to the chinch bug of the bentonite-organic base complex.

Table 3.—*Mortality from insecticides used as dusts in field tests on the chinch bug*

Insecticide	Application per acre	Date	Stage of insect	Estimated mortality, in—			Injury to corn plant
				2 hours	4 hours	24 hours	
	Pounds			*Percent*	*Percent*	*Percent*	
Calcium cyanide	14.5	June 13	Third and fourth instars.	75	50	40	None.
	51.4	do	do	95	80	75	Do.
	51.4	June 22	Fourth and fifth instars.	95	60	50	Slight.
	90.6	July 6	Adults and fifth instar.	90	80	60	Do.
	300.0	June 22	Fourth and fifth instars.	98	90	85	Severe.
Sodium fluoride (5 percent) in bentonite	12.7	June 13	Third and fourth instars.	5	5	5	None.
	25.1	do	do	5	5	5	Do.
Powdered derris root (5.70 percent rotenone.)	7.2	do	do	5	15	25	Do.
	25.1	do	do	10	25	75	Do.
Anabasine sulphate (5 percent) in bentonite	25.1	June 15	do	5	5	5	Do.
	33.2	do	do	5	5	5	Do.
Nicotine sulphate (5 percent) in bentonite	25.1	do	do	5	5	5	Do.
	42.3	do	do	5	5	5	Do.

DISCUSSION

The soaps which have been tested against the adult chinch bug gave approximate median lethal concentrations of 0.9 to 1.5 percent. These compare with about 0.2 percent of neutral sodium oleate for the bean aphid (*Aphis rumicis*), established by Tattersfield and Gimingham (*41*) and recently confirmed by Dills and Menusan (*6*), and 2.6 percent sodium oleate for the adult Japanese beetle (*Popillia japonica* Newm.) determined by Fleming and Baker (*9*). The latter value was obtained by a submersion method. In resistance to sodium oleate, the chinch bug appears to occupy an intermediate position between the bean aphid and the adult Japanese beetle.

The median lethal concentration of nicotine in aqueous solution for the bean aphid is about 0.04 percent (*40*). The value for the adult chinch bug is approximately 0.08 percent, indicating a greater resistance of this insect to nicotine.

The median lethal concentration of a mixture containing pyrethrins I and II for the chinch bug is about 0.0025 percent. Hartzell and Wilcoxon (*20*) have presented a mortality curve for the bean aphid which shows the relation between percentage kill and the total pyrethrin content of aqueous spray mixtures. The median lethal con-

centration read from this curve is close to 0.01 percent of total pyrethrins for aphids of various ages. Tattersfield, Hobson, and Gimingham (*42*) have determined the toxicity to the adult wingless bean aphid of both pyrethrin I and pyrethrin II. The median lethal concentration for the former (read from diagram 1–2) is about 0.0008 percent; for pyrethrin II it is much greater, about 0.0065 percent. Wilcoxon and Hartzell (*45*) have confirmed this large difference in toxicity to the bean aphid of pyrethrin I and pyrethrin II. Gnadinger (*18*) has discussed critically the published work on the relative toxicities of the pyrethrins, and suggests that pyrethrin II in aqueous suspensions may have little toxicity for aphids yet be nearly as toxic as pyrethrin I for cockroaches and (in light petroleum oil) for house flies. The necessity for further toxicity studies of the pyrethrins upon other species of insects is apparent.

Upon the basis of the figures of Tattersfield, Hobson, and Gimingham (*42*) suspensions of pyrethrins may be somewhat less toxic to the adult chinch bug than to the adult bean aphid, but close comparisons cannot be made from the data available.

Rotenone applied as a spray in acetone solution is singularly nontoxic to adult and fifth-instar chinch bugs. On the other hand, it is highly toxic to aphids (*5, 34*), exceeding the pyrethrins and nicotine in this respect. When applied to the body surface of insects, rotenone appears to be very selective in its action regardless of the criterion of effect.[4] Although exceedingly toxic as a stomach poison to the silkworm larva (*35*), it is almost without effect on grasshoppers (*31*). Blanket recommendations for the insecticidal use of rotenone are not justified from present knowledge of its toxicity.

SUMMARY

This paper reports the results of a laboratory and a field investigation to determine the effectiveness of certain contact insecticides against the chinch bug, *Blissus leucopterus* (Say). In the laboratory five soaps (sodium oleate, sodium-base laundry soap, pure potassium oleate, commercial potassium oleate, and commercial potassium fish-oil soap), two kerosene emulsions, nicotine, piperidine, pyrethrum extract, rotenone, and derris extracts were applied as sprays under standardized conditions to the various stages of the insect. In the field, three commercial soaps, several petroleum oil emulsions, nicotine sulphate, a preparation containing dipyridyls, pyrethrum extract, and rotenone and derris extracts were tested as sprays on chinch bugs in corn. Calcium cyanide, sodium fluoride, nicotine-bentonite mixture, anabasine-bentonite mixture, and powdered derris root were applied as dusts. The results of these experiments are as follows:

(1) Laboratory experiments: Laundry soap was the most toxic; among the other four soaps no significant differences were found. The adult bugs are generally more resistant than the younger forms. Kerosene emulsion at high oil concentrations was only mildly toxic; emulsions which contained naphthalene dissolved in the oil were more toxic. Nicotine was more toxic to the adults than to the younger stages. Piperidine was relatively nontoxic. Pyrethrum extract in dilute acetone solution was highly toxic on the basis of total pyrethrin

[4] SIMANTON, W. A. THE QUANTITATIVE TOXICITY OF FIVE TYPICAL CONTACT INSECTICIDES TO SEVERAL SPECIES OF INSECTS. 38 pp. 1935 (Unpublished thesis, Iowa State College.)

content and about equal to derris extract when the concentration of the latter was expressed as rotenone. On the above-mentioned bases both pyrethrum extract and derris extract were much more toxic than rotenone. The latter was almost nontoxic to adults and fifth-instar nymphs. There was no difference in toxicity between acetone and carbon tetrachloride extracts of derris.

(2) Sprays in the field: Laundry soap was no more effective than commercial potassium oleate or potassium fish-oil soap. The former was less injurious to the plant, suggesting that soaps of the higher saturated fatty acids (palmitic and stearic) may be less toxic to the corn plant. The alkalinity of the soaps may also be a factor. Nicotine and pyrethrum extracts gave results in substantial accord with those of the laboratory. Rotenone was less effective in the field than in the laboratory, as were also the derris extracts. Dipyridyl oil was noninjurious to the plant at effective concentrations.

(3) Dusts in the field: Calcium cyanide was efficient only when the amounts applied were large enough to cause severe plant injury. The other materials used were valueless.

The approximate order of toxicity in the laboratory of the various materials based upon the median lethal concentrations for the adults was: Pyrethrins=derris extract (based on rotenone content)>nicotine>sodium base laundry soap>other K and Na soaps>piperidine>kerosene-naphthalene emulsion>kerosene emulsion. Rotenone is not included because the toxicity data are insufficient.

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